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PROBLEM DEFINITION STUDIES ON POTENTIAL ENVIRONMENTAL POLLUTANTS

V. PHYSICAL, CHEMICAL, TOXICOLOGICAL, AND BIOLOGICAL PROPERTIES
OF SEVEN CHEMICALS USED IN PYROTECHNIC COMPOSITIONS

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U.S. Army Toxic and Hazardous Materials Agency
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) → This report provides a data base and summary of physical/chemical properties, analytical methods, mammalian toxicology, environmental considerations, and standards, where available, for: auramine; benzanthrone; 1,4-di-p-toluidinoanthraquinone; 1,4-diamino-2,3-dihydroanthraquinone; 1-methylaminoanthraquinone; Vat Yellow 4; and hexachloroethane; and identified information voids and recommends research needed to supply information for the adequate assessment of adverse health and environmental effects. →		

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Microorganisms	Standards
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Mutagenesis	Toxicity
Persistence	Translocation
Physical-chemical properties	Transport
Plants	Vat Yellow 4
Pollution	Volatilization
Pyrotechnic compositions	Wildlife
Reptiles	

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INTRODUCTION

The U.S. Army Toxic and Hazardous Materials Agency, formerly the office of the Project Manager for Chemical Demilitarization and Installation Restoration, has identified an initial list of substances (Table 1) requiring assessment because of their actual or potential presence in the environment outside the boundaries of Pine Bluff Arsenal (PBA), Pine Bluff, Arkansas. Prior to initiation of this problem definition study, each substance was carefully considered and a revised list (Table 1) was developed. The rationale for this revision is discussed below.

For DDT and its analogues, there is an overwhelming amount of information available in the literature and most of the pertinent data have been summarized in a number of recent review articles. Thiodiglycol and phosphorus have already been assessed in recent reports by Rosenblatt et al.²⁻⁴ and Dacre and Rosenblatt.⁵ Therefore, DDT and its analogues, thiodiglycol, and phosphorus were deleted from the initial list and will be addressed in a separate report. On the basis of information obtained during a site visit to PBA,⁶ Vat Yellow 4 and hexachloroethane were included in the revised list. Vat Yellow 4 has been used as a substitute for the yellow dye, auramine, for the past 20 years, and hexachloroethane has been used as a component of white smokes. The other substances on the initial list, including auramine, were retained because of their history of use at PBA.

The inhalation toxicology of colored smokes generated from the anthraquinone dyes and Vat Yellow 4, not addressed in this report, has been investigated by Owens and Ward, who also reviewed the earlier mammalian toxicology literature for the dyestuffs,⁷ and by Weeks and Yevich.⁸ The objective of the present study is to provide technical information on the physical, chemical, toxicological, and biological properties of seven substances used in pyrotechnic compositions, with emphasis on possible environmental effects. The potential for contamination of PBA by these substances is considered elsewhere.⁹

In assessing the seven substances on the revised list (all of which are components of pyrotechnic compositions), the organization of technical and professional personnel, the manual and computerized literature searches, and the information handling system were the same as those detailed in the initial report of this series.² For the most part, retrieved references were published before 1978.

TABLE 1. INITIAL AND REVISED LISTS OF POLLUTANTS AT PINE BLUFF ARSENAL

Initial List^a

DDT
Thiodiglycol
Phosphorus (white)
Auramine
Benzanthrone
1,4-Di-p-toluidinoanthraquinone
1,4-Diamino-2,3-dihydroanthraquinone
1-Methylaminoanthraquinone

Revised List^b

Auramine
Benzanthrone
1,4-Di-p-toluidinoanthraquinone
1,4-Diamino-2,3-dihydroanthraquinone
1-Methylaminoanthraquinone
Vat Yellow 4
Hexachloroethane

a. As provided in Reference 1.

b. Substances addressed in this report; see text for discussion of deletions and additions.

SUMMARY OF FINDINGS

The findings from this study are presented in detail for each pollutant substance in Appendixes A through G. Nomenclature, chemical formulas, physical properties, solubilities and chemical reactivities, production and use, analytical methods, mammalian toxicology, environmental considerations, and standards are summarized below.

PHYSICAL/CHEMICAL PROPERTIES

Table 2 presents the common names, CAS Registry Number, Colour Index Number, Toxic Substances List Number, and Wiswesser Line Notation for each of the seven compounds. These names and numbers serve to identify these compounds and to provide access to information in secondary literature sources. Other alternative names listed in each appendix may be useful in the interpretation of older literature.

Table 3 presents the molecular weight, molecular formula, and structural formula of each of the seven compounds. Examination of these data allows an organic chemist to estimate values for some of the important chemical parameters for which no literature values are reported. The dyes considered in this study, because of their vivid colors and powdery properties, impart recognizable coloration to surfaces and soils. Although the dyes have high melting points (Table 4), at which temperatures some decompose, they are sufficiently stable to be aerosolized. All of the compounds are insoluble in water (Table 5) and in alkaline media. The dyes are more soluble in alcohols, ethers, aromatic solvents, chlorinated solvents, and concentrated sulfuric acid.

PURITY OF SUBSTANCES

For pyrotechnic uses, these compounds as procured may contain unidentified impurities as high as 23%. Products of thermal degradation, formed during aerosolization, are essentially unidentified. The toxicities of the impurities or the thermal degradation products, relative to parent or specified pyrotechnic substance, are unknown. Because these pyrotechnic substances are of both foreign and domestic origin, the starting materials in the syntheses, which are probably present as impurities, are not readily determined. Current trade reports indicate that all hexachloroethane, for example, is imported.

ANALYTICAL METHODS

Analytical methodology is not well developed for most of these compounds, possibly because commercial applications have not required precise, low-level detection methods. Recent developments in liquid chromatography and gas chromatography make it possible not only to analyze the purity of these substances in bulk, but also to determine them quantitatively in environmental samples.

TABLE 2. SUMMARY OF NOMENCLATURE

C.I.S Registry No.	Colour Index No. ^a	Toxic Substances List No. (1977)	Wisbecker Line Notation
Auramine	492-80-8	41000	BY35060 IN1&R DYUMR DN1&1
Auramine hydrochloride	2465-27-2	---	BY35750 IN1&R DYUMR DN1&1 &GH
Benzanthrone	32-05-3	--	CX50750 L C6666 1A Q IVJ
1,4-Di-p-toluidino-anthraquinone	128-80-3	61565	CB57750 L C6666 GV IVJ DMR DI& GMR D1
1,4-Diamino-2,3-dihydro-anthraquinone	81-63-0	--	-- L C6666 BV IV CU GUJ DZ CZ
1-Methylaminoanthraquinone	82-38-2	60505	-- L C6666 BV IVJ DMI
Vat Yellow 4	128-66-5	59100	HO70300 L D6 B66 0666 2AB A JV UV&J
Hexachloroethane	67-72-1	--	K140250 GX5GXGG

^a. Color Index (formerly known as Rowe's Colour Index). 1965. Published by the Society of Dyers and Colorists and the American Association of Textile Chemists and Colorists, 2nd ed., Bradford, England and Lowell, MA.

TABLE 3. SUMMARY OF FORMULAS

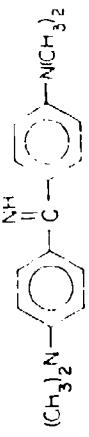
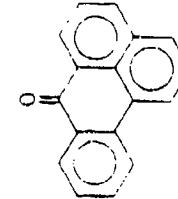
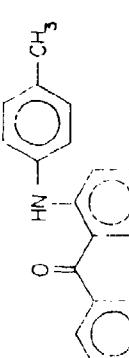
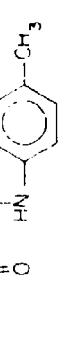
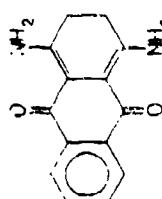
	Molecular Weight	Empirical Formula	Structural Formula
Auramine	267.41	$C_{17}H_{21}N_3$ (as base)	
Auramine hydrochloride	285.41	$C_{17}H_{22}ClN_3 \cdot H_2O$ (as hydrochloride)	
Benzanthrone	230.28	$C_{17}H_{10}O$	
1,4-Di-p-toluidino-anthraquinone	418.52	$C_{28}H_{22}N_2O_2$	
1,4-Diamino-2,3-dihydroanthraquinone	240.26	$C_{14}H_{12}N_2O_2$	

TABLE 3 (Cont.)

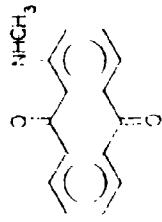
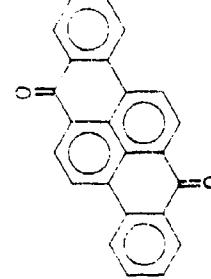
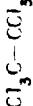
	Molecular Weight	Empirical Formula	Structural Formula
1-Methylanino-anthraquinone	237.11	C ₁₁ H ₁₁ NO ₂	
Vat Yellow 4	323.3	C ₂₄ H ₁₂ O ₂	
Hexachloroethane	236.70	C ₂ Cl ₆	

TABLE 4. SUMMARY OF PHYSICAL PROPERTIES

	Melting Point (°C)	Boiling Point (°C)	Density (g/mL)	Vapor Pressure (mm Hg)	Color
Curamine	136 (rapid heating)	--	--	--	Yellow
Benzanthrone	170-174	--	--	10 at 297.2°C 40 at 350.0°C	Pale yellow
				100 at 390.0°C 200 at 426.5°C	
1,4-Di-p-toluidino-anthraquinone	219.5-220.5	--	--	--	Green
1,4-Diamino-2,3-dihydro-anthraquinone	256 (decomposition)	--	--	--	Violet
1-Methylamino-anthraquinone	170	--	--	--	Red
Vat Yellow 4	385	--	--	--	Yellow
Hexachloroethane	186.9-187.4 (sealed tube)	186.8	2.091	1 at 20.0°C	White
	Sublimes without melting	184.4		3 at 32.7°C	

TABLE 5. SUMMARY OF SOLUBILITIES

	Solubility in Water	Solubility in Other Solvents
auramine	As a base, insoluble in water; as a hydrochloride, soluble in water	As a base, soluble in ethyl ether, very soluble in ethanol; as a hydrochloride, soluble in ethyl ether and glycerol and very soluble in ethanol and chloroform
Benzanthrone	Insoluble	0.52 g/100 g acetic acid 1.61 g/100 g benzene 2.05 g/100 g chlorobenzene Soluble in sulfuric acid
1,4-Di- <i>p</i> -toluidino- anthraquinone	Insoluble	3.4 g/100 mL alcohol at 25°C Soluble in hydrochloric, sulfuric, and other strong acids
1,4-Diamino-2,3-di- hydroanthraquinone	Insoluble	Soluble in glacial acetic acid and hot ethanol
1-Methylamino- anthraquinone	Insoluble	Soluble in acetone, chloroform, and cellosolve; slightly soluble in benzene and carbon tetrachloride
Var Yellow 4	Insoluble	Soluble in sulfuric acid, tetrahydro- naphthalene, xylene, and nitrobenzene; slightly soluble in acetone, benzene, ethyl alcohol, chloroform, o-chloropheno, pyridine, and toluene
hexachloroethane	50 mg/kg at 22°C	Soluble in alcohol, benzene, chloroform, ether, and oils

Hexachloroethane in aqueous samples can be analyzed by the purge and trap technique followed by gas chromatography and mass spectrometry (GC/MS).¹⁰ Quantitative analyses at the parts per billion level are possible. When lower precision is acceptable and/or GC/MS analysis is not economically justified, analysis can be carried out by gas chromatography or any of the classical techniques mentioned in Appendix G.

The polycyclic aromatic dyes, too unstable thermally for GC/MS analysis, can be characterized for purity by a combination of thin-layer column chromatography using several different solvent systems, differences in melting point, and ultraviolet (UV)-visible spectrophotometry. The most promising technique for the rapid quantitative analysis of environmental samples containing mixtures of polycyclic aromatic dyes appears to be liquid chromatography with visible or UV detection. These methods are not as well tested and documented as are the GC/MS methods for volatile organics; but rapid, quantitative analysis of anthraquinone dye mixtures has been demonstrated.¹¹

MAMMALIAN TOXICOLOGY

Toxicology data are summarized in Table 6. Occupational exposure studies and animal studies provide considerable information on auramine, benzanthrone, 1-methylaminoanthraquinone, and hexachloroethane, and some for Vat Yellow 4. Very little or no information is available for 1,4-di-p-toluidinoanthraquinone or 1,4-diamino-2,3-dihydroanthraquinone.

ENVIRONMENTAL CONSIDERATIONS

The literature provides no evidence of definitive studies on in vitro and in vivo fates of most of these materials. Their low solubilities in water indicate that they are present as particulate matter, as suspensoids, or in emulsoids. Only surface areas immediately adjacent to sites where grenades are manufactured or tested at PBA show recognizable soil or water colorations. It is not known whether the eventual disappearance of dyes is due to degradation or transformation, or to transport as particulate matter.

Literature data on environmental considerations are summarized in Table 7. Data are available for only three substances: auramine, benzanthrone, and hexachloroethane. No information was recovered concerning accumulation, transport, or degradation of any of the pyrotechnic substances.

STANDARDS

Benzanthrone standards have been reported for the Soviet Union. The occupational standard is 2 mg/m³ in air. A reservoir standard of 0.05 mg/kg has been suggested.

TABLE 6. SUMMARY OF TOXICOLOGICAL PROPERTIES

Substance	Toxicological Properties
Auramine	Causes bladder tumors in humans; hepatomas and lymphomas in mice; hepatomas and fibrosarcomas in rats; metaplasia of the urinary tract in rabbits.
Benzanthrone	Produces itching, erythema, skin pigmentation, pruritus, precocious generalized eczema, and photosensitization in humans. Causes liver damage, nervous system effects, and disturbances of the autonomic nervous system in humans. The oral LD ₅₀ for rats is 1.5 g/kg; for mice, 0.29 g/kg.
1,4-Di-p-toluidino-anthraquinone	Oral LD ₅₀ for rats is 3,060 mg/kg.
1,4-Diamino-2,3-di-hydroanthraquinone	Weakly mutagenic in <u>Salmonella typhimurium</u> (Ames Test)
1-Methylamino-anthraquinone	Skin irritant and sensitizer in humans. Slight toxicity observed in rats treated at 500 mg/animal for three days. Produces cystic changes in rat kidneys.
Vat Yellow 4	No definitive information retrieved on acute toxicology of Vat Yellow 4 per se. Commercial samples found to contain 0.1% dibenzochrysene, a potent carcinogen. A commercial formulation containing 18% of the dye caused an increased incidence of lymphomas in male mice when administered for 106 weeks in the food at 25,000 and 50,000 ppm.
Hexachloroethane	Chronic local irritant. Lowest lethal dose (subcutaneous) for rabbits is 4 g/kg. Oral LD ₅₀ for rat exceeds 4 g/kg. Oral doses of 1 g/kg cause central nervous system depression in dogs; 0.325 g/kg administered intravenously to dogs is lethal. Technical grade induces hepatocellular carcinomas in male and female mice administered, respectively, 1,179 and 590 mg/kg-day by gavage for 78 weeks.

TABLE 7. SUMMARY OF ENVIRONMENTAL CONSIDERATIONS

Substance	Environmental Considerations
Auramine	<p><u>Fish:</u> 5-day LC₅₀ is 1 ppm for fathead minnows.</p> <p><u>Invertebrates:</u> 0.05% in rearing medium causes mutagenic actions in <u>Drosophila melanogaster</u>.</p> <p><u>Microorganisms:</u> Inhibits growth of certain bacteria and fungi.</p> <p><u>Plants:</u> Produces mutagenesis and nuclear irregularities at low concentrations.</p>
Benzanthrone	<p><u>Invertebrates:</u> Toxic to <u>Daphnia</u> at 10 mg/L. Lethal to <u>Paramecium caudatum</u> at 0.2 mg/L.</p> <p><u>Microorganisms:</u> Induces cancerous growth in the marine alga <u>Porphyra tenera</u>.</p>
1,4-Di-p-toluidino-anthraquinone	No information was retrieved.
1,4-Diamino-2,3-dihydro-anthraquinone	No information was retrieved.
1-Methylamino-anthraquinone	No information was retrieved.
Vat Yellow 4	No information was retrieved.
Hexachloroethane	<p><u>Mammals:</u> In large doses, produces central nervous system depression. May produce chronic liver lesions, diffuse necrosis, and digestive disorders when used as an anthelmintic in cattle.</p> <p><u>Invertebrates:</u> Lethal to certain insects. Used for treatment of domestic animals infested with parasitic worms.</p> <p><u>Microorganisms:</u> At 5.3 mg/L, toxic to <u>Vibrio metchnikovii</u>.</p>

In the United States, a threshold limit value (TLV) for skin exposure to hexachloroethane has been set at 1 ppm or 9.7 mg/m³.

No standards or TLVs have been recommended for any of the other compounds.

DATA GAPS

Much information necessary for an evaluation of the environmental fate and effects of these seven substances was not retrieved from the literature. These data gaps are discussed below.

PHYSICAL/CHEMICAL PROPERTIES

Table 8 summarizes the information that was found on physical and chemical properties.

Physical Properties. The dyes are solids and as a group exhibit poor solubility in water, but these solubilities may be greatly changed in the presence of salts and detergents. These changes in solubility could play a major role in movement and biodegradation of these substances in the environment.

Chemical Properties. The dyes are sensitive to UV radiation and exhibit chemical changes in the presence of oxygen and other oxidizing agents. These chemical transformations have not been adequately documented to allow estimation of environmental stability.

Determination of Impurities. Dyes currently used in pyrotechnics contain impurities, the biochemical properties of which may or may not be known. Since impurities have not been qualitatively and quantitatively characterized, it is not possible to assign toxic properties to a specific substance.

Preparation of Pure Materials. Methods for the preparation of pure materials have not been adequately documented. This lack of documentation detracts from efforts to study physical, chemical, and biological properties. Similarly, the effects of handling and storage on purity are not documented.

Biochemical Transformations. The fate of hexachloroethane *in vivo* is known. Such data, however, are not currently available for the dyes. Studies of bioaccumulation, excretion routes, storage in vital organs, and degradation products are of importance in evaluation of environmental hazard.

MAMMALIAN TOXICOLOGY

Limited mammalian toxicity data exist for the seven compounds reviewed here. Table 9 summarizes data gaps for toxic properties.

TABLE 8. PHYSICAL/CHEMICAL DATA AVAILABLE

	Physical Properties (Solubilities)	Chemical Properties	Soil Movement	Preparation (Pure Material)	Determination of Impurities
Auramine	± ^a	±	-c	-	-
Benzanthrone	±	+ ^b	-	-	-
1,4-Di-p-toluidino-anthraquinone	±	±	-	-	-
1,4-Diamino-2,3-dihydroanthraquinone	±	±	-	-	-
1-Methylamino-anthraquinone	+	-	-	-	-
Vat Yellow 4	±	-	-	-	-
Hexachloroethane	+	+	-	+	+

a. ± = Information marginally adequate.

b. + = Sufficient information retrieved.

c. - = Insufficient information retrieved.

ECOLOGICAL EFFECTS

There is almost no information available concerning the toxicity of these substances to aquatic biota at the three major trophic levels (algae, invertebrates, and fishes). The phytotoxic and bioaccumulation properties of the pyrotechnic substances in common native plants grown in contaminated soil are not known. Bioaccumulation has not been studied.

ENVIRONMENTAL FATE

The environmental fates of these pyrotechnic materials are largely unknown. Because all except hexachloroethane have relatively low water solubilities and negligible volatility, their rates of dispersal in the environment may be low. The extent of microbial metabolism of the pyrotechnic materials is poorly documented. Thus, the extent of their degradation in the environment and the identities of possible stable metabolites are unknown.

TABLE 9. MAMMALIAN TOXICOLOGY DATA AVAILABLE

	Auramine	Benzan-throne	1,4-Di-P-toluidino-anthraquinone	1,4-Diamino-2,3-dihydro-anthraquinone	1-Methyl-amine-anthraquinone	Vat yellow 4	Hexa-chloro-ethane
Acute oral LD ₅₀ (rat/mouse) ^a	±2	+	+	-c	-	-	+
Acute dermal LD ₅₀ (rat/rabbit)	-	-	-	-	-	-	+
Eye and skin irritation (rabbit)	-	-	±	-	-	-	+
Skin sensitization (guinea pig)	-	+	-	-	-	-	+
Metabolism (various animals) ^d	-	-	-	-	-	-	+
Mutagenesis in microbes ^e	-	+	±	±	±	-	+
Chronic toxicity (oral dosing of mouse and rat)	±	-	-	-	-	-	±
Carcinogenicity testing (mouse, rat, and dog)	±	-	-	-	-	-	+
Reproduction studies (rat)	-	±	-	-	-	-	-
Teratology (rat)	-	-	-	-	-	-	+

a. ± = Information marginally adequate.

b. + = Sufficient information retrieved.

c. - = Insufficient information retrieved.

d. Metabolism includes absorption, distribution, excretion, and pharmacokinetics.

e. Ames test, including activation.

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APPENDIX A

AURAMINE

ALTERNATIVE NAMES

4,4'-Imidocarbonyl-bis(N,N'-dimethyl)benzenamine; 4,4'-(imidocarbonyl)-bis(N,N'-dimethyl)aniline; 4,4'-dimethylaminobenzophenonimide; bis(p-dimethylaminophenyl)methyleneimine; tetramethyl-p-diamino-imidobenzophenone; tetramethyldiaminodiphenylacetimine; apycrine auramine base; auramine N base; auramine O base; auramine SS; auramine OO; C.I. Basic Yellow 2 (free base); yellow pyoctanine; Glauramine.¹

PHYSICAL AND CHEMICAL PROPERTIES

CAS Registry No.: 492-80-8 (auramine); 2465-27-2 (auramine hydrochloride)²

Colour Index No.: 41000

Toxic Substances List No.: BY35000, BY36750

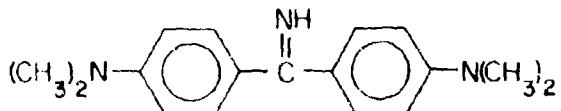
Wiswesser Line Notation: 1N1&R DYUMR DN1&1; 1N1&R DYUMR DN1&1 &GH²

Military Specifications: MIL-A-3664 (9 January 1952) for auramine hydrochloride³

Molecular Weight: 267.41 (base)

Empirical Formula: C₁₇H₂₁N₃ (base)

Structural Formula:



Physical properties of auramine are listed in Table A-1.

TABLE A-1. PHYSICAL PROPERTIES OF AURAMINE

Property	Value	Reference
Melting Point (Base)	136°C (rapid heating)	1
Boiling Point	-	
Density	-	
Vapor Pressure	-	
Color	Yellow	
Solubility in Water	As a base, insoluble in water; as hydrochloride, soluble in water	1
Solubility in Other Solvents	As a base, soluble in ethyl ether, very soluble in ethanol; as hydro- chloride, soluble in ethyl ether and glycerol and very soluble in ethanol and chloroform	1
Chemical Reactivity	Decomposes at temperatures above 70°C; is a weak base that forms salts with HCl and H ₂ SO ₄	1

PREPARATION

Auramine is manufactured industrially from dimethylaniline and formaldehyde, which react to form Michler's base (tetramethyldiaminodiphenylmethane). This base is subsequently converted to auramine by heating it with sulfur and ammonium chloride in the presence of ammonia.¹

PRODUCTION AND USE

Imports of auramine as Basic Yellow 2 totaled 113,255 pounds in 1973 and 105,400 pounds in 1974.^{4,5}

The free base of auramine is used to prepare Solvent Yellow 34, a soluble yellow dye. In the United Kingdom, auramine has been used as a powerful antiseptic in nose and ear surgery, and a specially purified auramine, sold under the name of Glauramine, is used as an antiseptic in the treatment of gonorrhoea. Auramine and its hydrochloride are used in large quantities in the coloring of paper and cardboard and, to a lesser extent, of some textiles and leather. In the first-mentioned case, they are added during the processing of the raw material prior to manufacture. Auramine has been used in some countries as a food dye and is also used as a smoke dye.¹

ANALYTICAL METHODS

Guidelines for the analysis of aromatic amines have been reported.⁶ A specialized reverse-phase liquid chromatography procedure, termed paired-ion chromatography, has been useful for the analysis of high molecular weight aromatic compounds not readily analyzed by gas chromatography.⁷ Ripley and Need have reported on analytical methods applicable to the analysis of auramine in smoke mixtures.⁸

MAMMALIAN TOXICOLOGY

Human Exposures

Anson and Parent⁹ have noted that injury to the human eye by auramine is characterized by conjunctivitis and keratitis; external application to the skin is said to have caused "severe inflammatory reactions, destruction of tissue with severe pain and fever, vomiting, headache, and yellow vision."

One study showed a relatively high incidence of bladder tumors in workers engaged in the manufacture of auramine. A latent period ranging from 9 to 28 years with an average of 19.3 years was observed, similar to that found for benzidine and 2-naphthylamine.¹⁰ Overall, six death certificates listed

bladder tumors among these workers; only 0.45 would have been expected based on the mean rate for the male population of England and Wales ($P < 0.005$). The morbidity was nine cases.

Experimental Animals

Parent has reviewed the literature prior to 1964 on the acute and chronic toxicities of auramine to experimental animals.¹¹ The oral LD₅₀ for administration of the hydrochloride to mice is reported to be 480 mg/kg.

Thirty mice were given a diet containing 0.1% commercial auramine for 52 weeks (total dose: 728 mg per animal). The animals were kept for their life spans. Seven hepatomas and 11 lymphomas were found compared with 0 and 5, respectively, in 60 control animals receiving weekly injections of arachis oil. Two other tumors were also reported.^{1,12} In one study, 30 stock mice and 27 CBA mice of both sexes were given diets containing auramine dissolved in acetone at concentrations of 0.1% and 0.2% (the maximum tolerated), respectively, for 52 weeks; approximate total doses were 1,820 mg and 3,650 mg per mouse, respectively. In stock mice, 57% of the males and 30% of the females that survived to tumor-bearing age showed hepatomas. No cholangiomas were observed, and the degree of cirrhosis was minimal. No hepatomas were seen in 16 control animals. In CBA mice (the high dose group), the frequency of hepatomas was 58% for males and 73% for females in the treated group compared with 11% and 5% for males and females, respectively, in the control group. A few other tumors occurred in stock mice, but none in CBA mice.^{1,13}

Twelve male Wistar rats were given a diet containing 0.1% commercial auramine for 87 weeks (estimated total dose: 10 g per rat). Ninety-two percent of the animals (11/12) developed hepatomas between weeks 91 and 122 following the start of treatment. A few other tumors were observed. Twelve control rats were tumor-free at death, which occurred between 90 and 120 weeks.^{1,13}

For 5 days per week for 21 weeks, 24 male Wistar rats were given subcutaneous injections of 0.1 mL per 100 g body weight of a 2.5% suspension of commercial auramine in arachis oil (estimated total dose: 110-120 mg per animal). In 20 animals that survived 21 weeks of treatment, 11 fibrosarcomas (55%) and 3 hepatomas (15%) were observed between 40 and 113 weeks. Three intestinal carcinomas were also reported.^{1,13}

In a preliminary comparative experiment, nine rabbits were given auramine (purity not stated) orally to the limit of tolerance, and the treatment was continued until the onset of the final illness. Six animals were sacrificed in the first 2 years and three between 3 and 4 years. Metaplasia of the urinary tract epithelium, suggestive of precancerous change, was seen in two out of five rabbits examined, compared with one of seven control rabbits.^{1,14,15}

No abnormalities have been detected in dogs given auramine orally (purity not stated) daily for about 7 years (total ingested amount: 66 g).^{1,16}

The above and some additional data are summarized in Table A-2. None of the retrieved material reports the induction of bladder tumors in experimental animals.

Biochemistry

No information was retrieved.

Mutagenicity

Auramine exhibits mutagenic actions in the fruit fly, Drosophila melanogaster, when added to the larval rearing medium at a concentration of 0.05%.²⁰

Carcinogenicity

Auramine has been included along with certain other carcinogenic compounds in the Carcinogenic Substances Regulations 1967²¹ of Great Britain. These regulations control the employment of persons in connection with the making of this substance.

ENVIRONMENTAL CONSIDERATIONS

Behavior in Soil and Water

Degradation. No information was retrieved, but auramine is the imine of 4,4'-bis(N,N-dimethylamino)benzophenone (Michler's ketone), to which it may degrade in water.

Animals

Mammals. No information was retrieved.

Birds. No information was retrieved.

Fish. Anson and Parent⁹ described the effect of auramine hydrochloride on fathead minnows. The 5-day LC₅₀ is given as 1 ppm; the 100-minute LC₅₀ is given as 10 ppm, and 0.1 ppm is said to have no effect over 13 days. Water quality parameters, such as hardness, temperature, dissolved oxygen, and pH, are unknown.

Reptiles. No information was retrieved.

Amphibians. Derouaux and Lecomte²² report that the oxidation-reduction potential of auramine is high enough to inhibit energy-producing metabolism in frog muscle. Concentrations of 0.05% and less produced spontaneous skeletal muscle contracting and arrest of the heart in systole.

TABLE A-2. TUMOR INDUCTION IN EXPERIMENTAL ANIMALS BY AURAMINE

Animal	No.	Route	Dose	Duration of Exposure	Tumor Occurrence	Reference
Mouse	30	Oral	0.1% in diet (14 mg/wk) for 52 wk	>90 wk	3M, 8F lymphoma 4M, 3F hepatoma	12
Mouse	60	sc	0.1 cc arachis oil (0.2 cc/wk) for 52 wk (control)	>90 wk	1M, 4F lymphoma	12
Mouse	17	Oral	120 mg in diet for 50-87 wk	89 wk	7 hepatoma	15
Mouse (CBA)	27	Oral	120 mg in diet		18 hepatoma	15
Mouse (CBA)		Control			11.2M, 5%F hepatoma	15
Mouse	15M	Oral	0.1% in diet for 52 wk (1,820 mg total)	89 wk	4/7 hepatoma; a few others	13
Mouse	15F	Oral	0.1% in diet for 52 wk (1,820 mg total)	89 wk	3/10 hepatoma	13
Mouse (CBA)	12M	Oral	0.2% in diet for 52 wk (3,640 mg total)	89 wk	7/12 hepatoma	13
Mouse (CEA)	15F	Oral	0.2% in diet for 52 wk (3,640 mg total)	89 wk	11/15 hepatoma	13
Mouse (CBA)	35M	Control		119 wk	4/35 hepatoma	13
Mouse (CEA)	55F	Control		119 wk	3/55 hepatoma	13
Mouse	8M	Control		109 wk	0/8 hepatoma	13
Mouse	8F	Control		109 wk	0/8 hepatoma	13
Mouse (C57 x IF)	12M	ig	1 mg/day in arachis oil	9 wk	3 mild bladder epithelial hyperplasia	17

TABLE A-2 (Cont.)

Animal	No.	Route	Dose	Duration of Exposure	Tumor Occurrence	Reference
Rat		Oral	In food		no tumors	18
Rat	12	Oral	Diet	90-129 wk	11 hepatoma, 1 other	15
Rat	20	sc	120 mg inj for 40-113 wk		11 sc fibrosarcoma, 3 hepatoma, 3 intestinal carcinoma	15
Rat	12M	Oral	0.1% in diet for 9 wk (360 mg total)	129 wk	0/12 hepatoma 90-129 wk	13
Rat	12M	Oral	0.1% in diet for 87 wk (10 g total)	129 wk	5/6 hepatoma 90-99 wk, 6/6 hepatoma 100-129 wk	13
Rat	12M		Control	129 wk	0/12 hepatoma	13
Rat	24M	sc	5 day/wk for 21 wk (110-120 mg total)	113 wk	3/20 hepatoma, 11/20 fibrosarcoma, a few others	13
Rat	20F	ig	Single dose, 150 mg/rat	6 mo	1/20 dead, no neoplastic change	19
Rabbit	9	Oral	"Fed to limit of tolerance"	3 yr	0 bladder tumors	15
Dog		Oral	Daily in feed (66 g total)	ca. 7 yr	"no abnormality"	16

Macroinvertebrates. No information was retrieved.

Microorganisms

Concerning fungi, auramine is reported to bring about complete inhibition of growth in Monilinia fructicola at 100 mg/L, but little or no inhibition at 25 mg/L in a basal medium at pH 5.3.²³ Czekalowski²⁴ studied the interaction of an Escherichia coli culture, a T2 bacteriophage, and auramine. Auramine at a concentration of 0.66×10^{-3} M produced no growth change in E. coli but produced growth inhibition of the phage. At 0.066×10^{-3} M, the phage was killed, but the host grew normally.

Auramine is claimed to be effective against wood-destroying fungi at 100 mg/L.²⁵ Measured bacteriostatic effects of auramine range from complete inhibition to no inhibition (Table A-3) depending on the organism tested.

Plants

Auramine, in concentrations of 5×10^{-5} M, when applied to the root tip of Allium and exposed to light or darkness, did not show any mutagenic activity.²⁰ However, another study reported that auramine, in concentrations of 10^{-4} M, applied for 10 or 20 minutes in darkness or under light, caused significant mutagenesis in Vicia faba.²⁰ Concentrations of auramine in the range of 20×10^{-6} M to 1×10^{-6} M, when applied to Allium root tip, caused hypertrophy with abnormal mitosis and appearance of binuclear cells.³⁰ In Tradescantia virginica, auramine induced formation of chromonema bridges.³⁰

Food Chain

No information was retrieved.

EXISTING STANDARDS

No information was retrieved.

TABLE A-5. BACTERIOSTATIC ACTIVITY OF AURAMINE

Species	Medium	Auramine Concentration (mg/L)	Effect	Reference
<u>Streptococcus faecalis</u>	Nutrient broth	25.0	Complete growth inhibition	26
<u>Streptococcus faecalis</u>	Nutrient broth	12.5	No growth inhibition	26
<u>Staphylococcus aureus</u>	Broth	25.0	Complete growth inhibition	26,27
<u>Staphylococcus aureus</u>		12.5	No growth inhibition	26,27
<u>Staphylococcus aureus</u>	Nutrient broth	62.5	Minimum inhibitory conc.	28
<u>Proteus vulgaris</u>	Nutrient broth	62.5	Minimum inhibitory conc.	28
<u>Salmonella typhi</u>	Nutrient broth	125.0	Minimum inhibitory conc.	28
<u>Klebsiella pneumoniae</u>	Nutrient broth	31.2	Minimum inhibitory conc.	28
<u>Bacillus mycoides</u>	Nutrient broth	15.6	Minimum inhibitory conc.	28
<u>Pseudomonas aeruginosa</u>	Nutrient broth	>500.0	Minimum inhibitory conc.	28
<u>Clostridium tetani</u>	Nutrient broth	31.2	Minimum inhibitory conc.	28
Lactic acid bacteria	Skim milk	3,000.0	46% inhibition of lactic acid production	29

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APPENDIX B
BENZANTHRONE

ALTERNATIVE NAMES

7H-Benz(de)anthracen-7-one (C.A. after 1936); benzanthrone (C.A. after 1936); 1,9-benzanthrone; benzanthrenone; mesobenzanthrone.

PHYSICAL AND CHEMICAL PROPERTIES

CAS Registry No.: 82-05-3

Colour Index No.: None

Toxic Substance List No.: CX50750¹

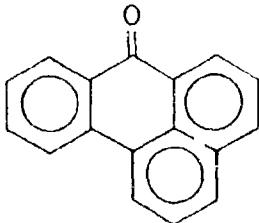
Wiswesser Line Notation: L C6666 1A Q IVJ¹

Military Specification: MIL-D-50074D²

Molecular Weight: 230.28

Empirical Formula: C₁₇H₁₀O

Structural Formula:



Physical properties of benzanthrone are listed in Table B-1.

TABLE B-1. PHYSICAL PROPERTIES OF BENZANTHRONE

Property	Value	Reference
Melting Point (Base)	174°C (rapid heating) 174.1°C 170°C	3 4 5,6
Boiling Point	-	
Density	-	
Vapor Pressure	10 mm (297.2°C) 40 mm (350.0°C) 100 mm (390.0°C) 200 mm (426.5°C)	3 3 3 3
Color	Pale yellow	3
Crystalline Form	Yellow orthorhombic needles when crystallized from ethyl alcohol, xylene, benzene, or nitrobenzene	3
Heat of Sublimation	-	
Solubility in Water	Insoluble	6
Solubility in Other Solvents	0.52 g/100 g acetic acid 1.61 g/100 g benzene 2.05 g/100 g chlorobenzene Soluble in sulfuric acid	6 6 6 6
Temperature of Decomposition	426°C	6

PREPARATION AND PURIFICATION

Benzanthrone is prepared by several methods, but most commonly by the condensation of reduced anthraquinone with glycerol in the presence of sulfuric acid at 135°C.⁷⁻⁹ The anthraquinone is reduced with copper or iron salts or with aniline sulfate. Another common industrial preparation is the ring closure of phenyl α -naphthyl ketone, which is dehydrogenated with AlCl₃, FeCl₃, or sodium m-nitrobenzenesulfonate.⁸ Other, less important syntheses are the thermal degradation of cinnamalanthrone in the presence of sodium-aluminum chloride¹⁰ and the dehydration of 1-phenylnaphthalene-2-carboxylic acid.

Depending upon the method of synthesis, the impurities may be numerous and toxic. Some impurities obtained are anthracene, anthraquinone, ferric, copper, and aluminum ions, acrolein, and glycerol; intermediates, by-products, and derivatives of benzanthrone are also found.

Benzanthrone is purified by vacuum sublimation with subsequent recrystallization from ethyl alcohol and/or from xylene.¹¹ A high pressure, preparative, liquid chromatography method has been developed that promises to give pure benzanthrone.¹² The UV absorbance at 513 nm is used to estimate its purity.²

PRODUCTION AND USE

In 1973, more than 1,086,000 pounds of benzanthrone were produced in the United States.¹³ This quantity was produced *in toto* by American Color and Chemical Corp., American Cyanamid Co., E.I. Du Pont de Nemours, Inc., GAF Corp., Otto B. May, Inc., Martin Marietta Corp., and Toms River Chemical Corp. Suppliers of dyes to PBA are Atlantic Chemical Manufacturing Corp., Carey Industries, American Aniline Products, and GAF Corp. In 1974 and 1975, 838,000 and 374,000 pounds of benzanthrone, respectively, were produced in the United States.^{14,15} In 1974, benzanthrone imports totaled 24,808 pounds.¹⁶

Benzanthrone, a yellow compound devoid of dyeing character, is used primarily as a starting material for the manufacture of dyes that have excellent fastness to light, chlorine, and washing.¹⁷ It is used in both green and yellow smokes.¹⁸ Its exceptional stability contributes to overall aerosolization efficiencies of about 58% in green smokes and 73% in yellow smokes.¹⁹

CHEMISTRY

Benzanthrone dissolves in sulfuric acid to form an orange solution with green fluorescence.⁶ Although it decomposes at its melting point, sufficient material volatilizes in a smoke mixture to produce a yellow or green smoke efficiently. In the tree smoke dyes, the percentage of benzanthrone present in the resulting aerosols is about 13.5% higher in green smoke and

8.5% higher in yellow smoke than in the initial mixes of benzanthrone from which the aerosols were made.¹⁹ These increases suggest greater thermal stability of benzanthrone than Solvent Green 3 in green smoke and HVT Golden Yellow (Vat Yellow 4) in yellow smoke.

The observation that flash photolysis of benzanthrone in hydrogen-donating solvents saturated with oxygen does not result in product formation at natural pH values²⁰ suggests that this compound will not be readily degraded photochemically in the environment. Benzanthrone is readily attacked by both nucleophilic and electrophilic reagents.^{7,8}

ANALYTICAL METHODS

Thin-layer chromatography has been used extensively to detect benzanthrone.²¹⁻²³ Typical stationary phases include alumina G and cellulose. Typical moving phases are pentane:ether (19:1), toluene, and dimethylformamide:water (35:65). Detection is by UV-excited visible fluorescence. Absorption maxima occur at 250, 282, 303, 387, and 513 nm.²⁴ A recent method for estimation of benzanthrone in drinking water achieves a detection limit of 125 pg (about 0.1 ng/L) by preconcentration on C₁₈-bonded pellicular packing followed by desorption and analysis by liquid chromatography, using fluorescence detection.²⁵

Benzanthrone has also been estimated polarographically²⁶ and in blood by spectrofluorometric methods.²⁷

MAMMALIAN TOXICOLOGY

Human Exposures

Benzanthrone is reported variously to cause an itching, burning sensation, erythema, dermatitis, and skin pigmentation.²⁸⁻³³ In sensitive individuals, actinic dermatitis or leukoderma can develop due to a photodynamic effect.²⁹⁻³⁵ Pruritus, precocious generalized eczema, pigmentation, and photosensitization in workers exposed to benzanthrone have been observed.³³ Systemic effects result from liver damage,³⁶ nervous system damage,³⁷ and disturbance of the autonomic nervous system regulatory function. Because of the toxic character of this dye, the U.S. Army Environmental Hygiene Agency³⁸ advised substituting a less toxic chemical for benzanthrone in smoke mixtures.

Experimental Animals

Benzanthrone did not produce signs of intoxication in male and female rats in oral doses up to and including 7.1 g/kg.⁴⁰ It was found to be nonirritating to the skin of the albino rabbit, for which the acute dermal LD₅₀ exceeded 3,000 mg/kg.⁴⁰ The dye was not irritating when applied to clipped, intact, or abraded skin of guinea pigs for 24 hours during a skin contact experiment.^{41,42} One report⁴⁰ found no irritation of the eye.

due to benzanthrone, whereas another report⁴³ noted moderate erythema, slight chemosis of the lids, lacrimation, and a light corneal stain. No indication of photoallergy in guinea pigs or phototoxicity in mice and swine was observed,⁴⁶ although others have stated that albino mice exhibit slight thickening and roughness of the skin on application of benzanthrone only in the presence of sunlight.⁴⁴

Studies of subacute oral intoxication have been carried out by intragastric introduction of benzanthrone doses equivalent to one half the intraperitoneal LD₅₀ (1.5 g/kg for rats and 0.29 g/kg for mice) once per week over a period of one month.⁴⁵ For rats, such treatment results in significant reduction in body and organ weight gain and some changes in blood parameters.

Five days after a single intraperitoneal injection of benzanthrone (200 mg/kg in arachis oil), all of four white male rabbits developed mild vascular congestion in the lamina propria and submucosa of the urinary bladder.⁴⁶ The urinary bladders of guinea pigs treated with single intraperitoneal injection of benzanthrone (25 mg/kg body weight), which were examined 7 days after injection, showed mild vascular congestion in the lamina propria and submucosa.⁴⁷ At 15 days, 6 of 10 guinea pigs showed evidence of urinary bladder mucosal lesion, severe vascular congestion, and a large number of inflammatory cells in the lamina propria and submucosa. Large areas of guinea pig lungs were filled with edematous hemorrhagic fluid within 24 hours after intratracheal injection of benzanthrone suspension (particle size <5 µg).⁴⁸

When benzanthrone (50 mg/kg body weight) suspended in distilled water was daily administered intraperitoneally to rats, normocytic anemia, probably of hemolytic origin, was observed at 10 and 20 days.⁴⁹ Biweekly intraperitoneal injection of benzanthrone (25 mg/kg) for 6 months leads to damage of the gametogenic function of rat testis, but not of the androgenic potency.⁵⁰ Testicular hyaluronidase activity was decreased, and treated rats showed patchy degeneration of the seminiferous tubules.

Significant decreases were noted in the level of ascorbic acid after 7 days in the blood, kidneys, and liver of adult male guinea pigs administered benzanthrone intraperitoneally at a dose of 25 mg/kg body weight.⁵¹ Intraperitoneal doses of 50 mg/kg-day to male rats resulted in a significant increase in plasma fibrinogen and a decrease in blood coagulation time.⁵²

Biochemistry

In a group of four rabbits, 26.1 to 30.5% of intraperitoneally administered benzanthrone was excreted unchanged in the urine in 5 days, more than half of which was excreted in the first 2 days.⁴⁶ The fate of the majority of the material is unknown.

Carcinogenicity

Available data are conflicting concerning benzanthrone carcinogenicity.⁴¹ In an early study, white mice (sex not given) received five subcutaneous injections, 10 to 15 days apart, of 0.5% benzanthrone in olive oil. Sixteen of the 32 mice survived after 6 months; one developed lung tumor, and one developed jaw tumor.^{53, 54} Results did not suggest that benzanthrone has tumorigenic properties.

Tests for the production of skin cancer have been carried out by applying 0.3 g of benzanthrone in solution (benzene?) to the interscapular region of mice twice weekly.⁵⁵ No epitheliomas or papillomas were observed on 10 mice so treated, of which the oldest survived 294 days.

Parent⁴¹ reported that this dye did not induce tumors in mice.

Mutagenicity

Benzanthrone was nonmutagenic when screened for mutagenicity with five Salmonella typhimurium tester strains with and without mammalian microsomal activation.⁵⁶ After exposure to ^{60}CO gamma irradiation in air, benzanthrone exhibits positive mutagenicity with strains TA1538 and TA98, which detect frameshift mutations.⁵⁷

Escherichia coli remained unaffected by benzanthrone in concentrations of 1 mg/mL. Bond and Gilleland⁵⁸ tested the ability of several compounds to produce changes in the RNA/DNA ratio in E. coli as a measure of relative carcinogenicity. Benzanthrone produced no alteration in the RNA/DNA ratio, indicating that it is nonmutagenic in E. coli.

Epstein et al.⁵³ conducted a dominant lethal mouse assay for benzanthrone-induced mutations. Male ICR/Ha Swiss mice were given single intraperitoneal injections of benzanthrone at doses of 1,000, 1,500, or 2,000 mg/kg. Two males (given 1,000 and 2,000 mg/kg) died. Remaining males were mated to three untreated virgin female mice per week for 3 or 8 weeks after the injection. Early fetal deaths and preimplantation losses were not produced by benzanthrone beyond control limits.

ENVIRONMENTAL CONSIDERATIONS

Production facilities for large quantities of benzanthrone are probably the primary source of human exposure to benzanthrone. Sites of grenade manufacture and testing at U.S. Army arsenals constitute the second largest source of exposure, and sites of grenade use or disposal constitute a third and important exposure source, particularly for military personnel.

Behavior in Soil and Water

Degradation. Loshakov⁶⁰ observed that benzanthrone, in concentrations above 0.05 mg/L, gives water a yellow color, and at 50 mg/L its odor becomes noticeable. Biological oxygen demand (BOD) is inhibited up to 13% by benzanthrone concentrations of 50 to 1,000 mg/L.

Animals

Mammals. No information was retrieved.

Birds. No information was retrieved.

Fish. No information was retrieved.

Reptiles. No information was retrieved.

Amphibians. No information was retrieved.

Invertebrates. Benzanthrone is toxic to Daphnia at 10 mg/L but not at 5 mg/L.⁶⁰ Epstein et al.⁶¹ used the toxicity of benzanthrone to the protozoan Paramecium caudatum as a measure of photodynamic activity. Concentrations of 0.21 μ g/mL produced 90% lethality in 30 minutes to dark incubated, motile ciliates exposed to 360-m μ irradiation at 8,750 μ watts/cm². Similar experiments have been performed with the nauplii of Artemia salina.⁶²

Plants

Ishio et al.⁶³ have shown that benzanthrone in coal chemical wastes induces cancerous growth in the edible marine alga, Porphyra tenera. Under laboratory conditions, benzanthrone at 2.0 mg/L induced algal cancer in all leaves of P. tenera within 40 days at 10°C. A later paper reported that benzanthrone (as well as some known carcinogens) brought about mitotic delays in Gyrodinium species.⁶⁴

Food Chain

No information was retrieved.

EXISTING STANDARDS

The USSR proposed occupational standard is <0.002 mg/L in air.³⁴ Loshakov suggested 0.05 mg/kg as the highest permissible concentration for reservoir water in the USSR.⁶⁰

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APPENDIX C

1,4-DI-p-TOLUIDINOANTHRAQUINONE

ALTERNATIVE NAMES

1,4-bis [(4-methylphenyl)amino]-9,10-anthracenedione (C.A. from 1972);
1,4-di-p-toluidino-9,10-anthracenedione (C.A. before 1972);
1,4-di-p-tolylamino-anthraquinone; C.I. Solvent Green 3; Quinizarine Green G
Base; D and C Green No. 6.

PHYSICAL AND CHEMICAL PROPERTIES

CAS Registry No.: 128-80-3

Colour Index No.: 61565

Toxic Substances List No.: CB57750¹

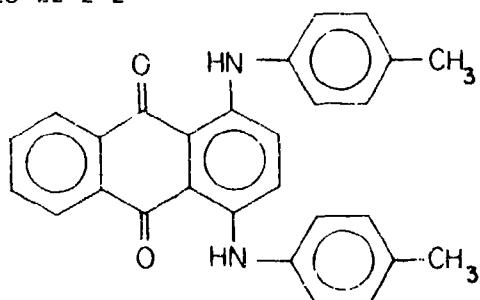
Wiessner Line Notation: I. C666 GV IVJ DMR DI& GMR D1¹

Military Specification: MIL-D-3277C²

Molecular Weight: 418.52

Empirical Formula: C₂₈H₂₂N₂O₂

Structural Formula:



Physical properties of 1,4-di-p-toluidinoanthraquinone are listed in Table C-1.

TABLE C-1. PHYSICAL PROPERTIES OF 1,4-DI-*p*-TOLUIDINOANTHRAQUINONE

Property	Value	Reference
Melting Point	219.5°-220.5°C	3
Boiling Point	--	
Density	--	
Vapor Pressure	--	
Color	Deep blue-violet (from glacial acetic acid)	4
Crystalline Form	--	
Heat of Sublimation	--	
Solubility in Water	Insoluble	5
Solubility in Other Solvents	Soluble in benzene and toluene; slightly soluble in acetone, alcohol, and ethyl acetate. Soluble in hydrochloric, sulfuric, and other strong acids	5

PREPARATION AND PURIFICATION

1,4-Di-p-toluidinoanthraquinone is prepared industrially by the condensation of p-toluidine with leucoquinizarin,^{6,7} anthraquinone-1,4-disulfonic acid,⁷⁻⁹ and 1,4-dichloroanthraquinone.^{6,9,10} Impurities in the dyes may be many, depending upon the method of synthesis; p-toluidine and anthraquinone starting materials as well as derivatives and isomers are probably present as contaminants. Because the sulfonic acid derivatives are made by using mercury salts, mercury may be a contaminant. Methods have been developed for removal of mercury when present in levels below 5 ppm.¹⁰

Purification of 1,4-di-p-toluidinoanthraquinone to meet Food and Drug Administration requirements for a drug dye can be achieved by repeated recrystallizations. For such use, the dye must contain no more than 0.5% impurities, 2% volatile matter at 135°C, and 1.5% carbon tetrachloride extractables; it must melt above 210°C.¹¹ Within these limits, the concentrations of impurities vary considerably; a study on the purification of aminoanthraquinones has shown that dyes with as much as 2% impurity give essentially the same melting point and UV-visible spectra with subsequent recrystallizations.¹²

It is now recognized that these physical measurements, to be meaningful, must be done in series with chromatographic separations. Materials that give a single band in several chromatographic solvent systems and a constant melting point or UV-visible spectrum are considered to be pure and suitable for biological testing. There are no available data that show that biological testing has been done with highest purity 1,4-di-p-toluidinoanthraquinone.

PRODUCTION AND USE

The primary commercial use of 1,4-di-p-toluidinoanthraquinone is in the textile industry, both as a dye and as an intermediate. It has been listed for use as a drug and cosmetic dye for sutures. Relatively large quantities are used for the manufacture of green smoke grenades. A typical pyrotechnic mixture contains a smoke-producing agent (1,4-di-p-toluidinoanthraquinone), an oxidizer (KC1O₃), a retardant (NaHCO₃), and sulfur. The grenade contains about 320 g of dye and will burn for approximately 70 to 80 sec/inch, during which time the dye vaporizes and is airborne.

In 1973, the United States produced 217,000 pounds and imported 4,760 pounds of 1,4-di-p-toluidinoanthraquinone as Solvent Green 3.^{13,14} The U.S. production figures of 1,4-di-p-toluidinoanthraquinone as D and C Green No. 6 are not available. However, U.S. production of the D and C green dyes in general was as follows: 39,000 pounds in 1973, 48,000 pounds in 1974, 23,000 pounds in 1975, and 18,000 pounds in 1976.¹⁵⁻¹⁷

CHEMISTRY

During combustion, 1,4-di-p-toluidinoanthraquinone decreases from 61% of the dye in an initial smoke grenade mix to 51% of the dye in the aerosol. This decrease suggests extensive thermal degradation of 1,4-di-p-toluidinoanthraquinone or relatively less volatilization than for benzanthrone, which increases in percentage in the mix during aerosolization.⁴

ANALYTICAL METHODS

1,4-Di-p-toluidinoanthraquinone is a derivative of anthraquinone and therefore can be detected by UV-visible and fluorometric methods. Maximum UV-visible absorption occurs at wavelengths of 402 nm ($\epsilon = 0.65 \times 10^4$), 550 nm ($\epsilon = 0.71 \times 10^4$), 600 nm ($\epsilon = 1.54 \times 10^4$), and 638 nm ($\epsilon = 1.64 \times 10^4$).

Separations have been achieved by chromatographic methods, especially thin-layer chromatography (TLC).¹⁸ Absorbents for TLC have been cellulose, magnesia, silica gel, and florisil.¹⁹ Some solvent systems that have been successfully used are acetone:hexane (1:3), pentane:ethyl ether, toluene, and dimethylformamide:water (35:65). Large-scale (gram) separations of 1,4-di-p-toluidinoanthraquinone have been carried out using column chromatography.²⁰ A recent study indicates that high pressure liquid chromatography (HPLC) is useful for detection and purification.²¹

MAMMALIAN TOXICOLOGY

Human Exposure

No information was retrieved.

Experimental Animals

The oral LD₅₀ for male and female rats is variously reported as 3,060 mg/kg,¹ greater than 10 g/kg,²² and greater than 15 g/kg.²³ The oral LD₅₀ for rats has been found to exceed 10 g/kg, with the minimum lethal dose greater than 6 g/kg.²⁴ This compound is said to produce no discernible skin irritation when applied to the intact and abraded skin of rabbits, and the minimum lethal dose by skin absorption is said to be greater than 8 g/kg (animal unspecified).²⁵ It produced "minimal" erythema when instilled into the conjunctival sac of rabbits.²⁴

Biochemistry

No information was retrieved.

Carcinogenicity

No information was retrieved.

Mutagenicity

1,4-Di-p-toluidinoanthraquinone was nonmutagenic when screened for mutagenicity with five Salmonella typhimurium tester strains with and without mammalian microsomal activation.²⁵

ENVIRONMENTAL CONSIDERATIONS

Behavior in Soil and Water

Degradation. No information was retrieved, but it should be noted that anthraquinones are readily reduced to the corresponding diols.

Animals

Mammals. No information was retrieved.

Birds. No information was retrieved.

Fish. No information was retrieved.

Reptiles. No information was retrieved.

Amphibians. No information was retrieved.

Invertebrates. No information was retrieved.

Microorganisms

No information was retrieved.

Plants

No information was retrieved.

Food Chain

No information was retrieved.

EXISTING STANDARDS

No information was retrieved.

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APPENDIX D

1,4-DIAMINO-2,3-DIHYDROANTHRAQUINONE

ALTERNATIVE NAMES

1,4-Diamino-2,3-dihydro-9,10-anthracenedione (C.A. from 1972);
1,4-diamino-2,3-dihydroanthraquinone (C.A. from 1972);
leuco-1,4-diaminoanthraquinone.

PHYSICAL AND CHEMICAL PROPERTIES

CAS Registry No.: 81-63-0

Colour Index No.: None

Toxic Substances List No.: None

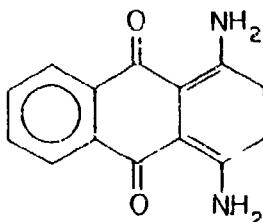
Wiswesser Line Notation: L C666 BV IV CU GUJ DZ GZ

Military Specification: MIL-D-3668¹

Molecular Weight: 240.26²

Molecular Formula: C₁₄H₁₂N₂O₂

Structural Formula:



Physical properties of 1,4-diamino-2,3-dihydroanthraquinone are listed in Table D-1.

PREPARATION AND PURIFICATION

1,4-Diamino-2,3-dihydroanthraquinone is readily prepared by the condensation of ammonia with 2,3-dihydroquinizarin (leucoquirizarin) in the presence of boric acid.³⁻⁵ Samples have been prepared on a small scale by the reduction of 1,4-diaminoanthraquinone.⁶

TABLE D-1. PHYSICAL PROPERTIES OF 1,4-DIAMINO 2,3-DIHYDROANTHRAQUINONE

Property	Value	Reference
Melting Point	256°C (decomposition)	2
Boiling Point	-	
Density	-	
Vapor Pressure	-	
Color	Violet	2
Crystalline Form	-	
Heat of Sublimation	-	
Solubility in Water	Insoluble	
Solubility in Other Solvents	Soluble in glacial acetic acid and hot ethanol	2

PRODUCTION AND USE

1,4-Diamino-2,3-dihydroanthraquinone is not as stable as many other anthraquinone dyes, but is an important intermediate in the synthesis of amino and leuco dyes. It is 80% of the dye used in the fabrication of violet smoke grenades.²

In 1973, U.S. manufacturers acknowledged the production of 890,000 pounds of 1,4-diamino-2,3-dihydroanthraquinone.⁷ Import quantities totaled 19,089 pounds.⁸ In 1974, U.S. production of 1,4-diamino-2,3-dihydroanthraquinone totaled 834,000 pounds and import quantities totaled 28,319 pounds.^{9,10} U.S. production of 1,4-diamino-2,3-dihydroanthraquinone for 1975 and 1976 was 470,000 and 531,000 pounds, respectively.^{11,12}

CHEMISTRY

1,4-Diamino-2,3-dihydroanthraquinone is a reduced diaminoanthraquinone and is easily oxidized by nitrobenzene.⁴ In strong bases, it is either readily oxidized by air to the anthraquinone dye^{4,5} or it loses ammonia, reverting to the dihydroxy leucoquinizarin.⁴ 1,4-Diamino-2,3-dihydroanthraquinone undergoes the general reactions of anthraquinones; mild conditions usually are sufficient for these reactions to occur.

ANALYTICAL CHEMISTRY

Percent purity of 1,4-diamino-2,3-dihydroanthraquinone used in pyrotechnic formulations is estimated spectrophotometrically at 456 \pm 2 nm. Ninety-five percent ethanol is used as the solvent and a pure sample is used as the reference standard. Potentiometric methods were unsatisfactory for quantitative estimation.¹³ Column, thin-layer, and paper chromatographic methods have been developed for 1,4-diaminoanthraquinone, but have not been applied to 1,4-diamino-2,3-dihydroanthraquinone. This omission is probably due to the fact that 1,4-diamino-2,3-dihydroanthraquinone is the intermediate most commonly used for 1,4-diaminoanthraquinone. There are no apparent reasons why the chromatographic techniques used for the 1,4-diamino dye cannot be used for this compound. UV-visible spectroscopy has been used successfully to analyze for the presence of this dye.¹⁴ Fluorescence and high pressure liquid chromatography should be equally applicable to the detection and estimation of 1,4-diamino-2,3-dihydroanthraquinone. 1-Amino-4-hydroxyanthraquinone, an oxidation product and contaminant of 1,4-diamino-2,3-dihydroanthraquinone, can be estimated spectrophotometrically at 603 nm by differential absorbance.¹⁵

MAMMALIAN TOXICOLOGY

No data were retrieved from the literature concerning the human exposures, experimental animal toxicology, biocompatibility, or carcinogenic potential of this dye or its impurities.

Mutagenicity

1,4-Diamino-2,3-dihydroanthraquinone was screened for mutagenicity with five Salmonella typhimurium tester strains with and without mammalian micro-somal activation.¹⁶ This dye is weakly mutagenic in strain TA1537 (frame-shift) whether or not activated by rat liver microsomes; it is nonmutagenic in other strains at low concentration and toxic at high concentration.

ENVIRONMENTAL CONSIDERATIONS

Behavior in Soil and Water

Degradation. No information was retrieved, but a reasonable degradation sequence would involve oxidation to an anthraquinone followed or accompanied by reduction to the corresponding diol. The most likely initial product, 1,4-diaminanthraquinone (Disperse Violet 1) has been estimated to be 10% degraded after 15 days aeration with domestic sewage microorganisms.¹⁷

Animals

Mammals. No information was retrieved.

Birds. No information was retrieved.

Fish. No information was retrieved.

Reptiles. No information was retrieved.

Amphibians. No information was retrieved.

Invertebrates. No information was retrieved. However, solutions of 1,4-diaminoanthraquinone, a likely degradation product of 1,4-diamino-2,3-dihydroanthraquinone, are reported to kill earthworms in 15 to 30 minutes at a concentration of 500 mg/L and in less than 20 hours at a concentration of 50 mg/L.¹⁸

Microorganisms

No information was retrieved.

Plants

No information was retrieved.

Food Chain

No information was retrieved.

EXISTING STANDARDS

No information was retrieved.

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APPENDIX E

1-METHYLAMINOANTHRAQUINONE

ALTERNATIVE NAMES

1-(Methylamino)-9,10-anthracenedione (C.A. from 1972); 1-N-methylamino-9,10-anthraquinone (C.A. before 1972); C.I. Disperse Red 9.

PHYSICAL AND CHEMICAL PROPERTIES

CAS Registry No.: 82-38-2

Colour Index No.: 60505

Toxic Substances List No.: None

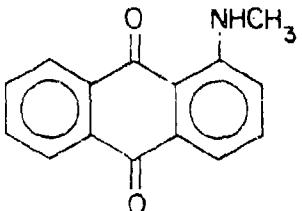
Wiswesser Line Notation: L C666 BV IVJ DM1

Military Specification: MIL-D-3284C¹

Molecular Weight: 237.11

Empirical Formula: C₁₅H₁₁NO₂

Structural Formula:



Physical properties of 1-methylaminoanthraquinone are listed in Table E-1.

TABLE E-1. PHYSICAL PROPERTIES OF 1-METHYLAMINOANTHRAQUINONE

Property	Value	Reference
Melting Point	169.5°C	2
Boiling Point	-	
Density	-	
Color	Red (from CCl_4) Deep red orange (from glacial acetic acid)	2 3
Crystalline Form	-	
Heat of Sublimation	--	
Heat of Vaporization	-	
Solubility in Water	Insoluble	
Solubility in Other Solvents	Soluble in acetone, chloroform, and cellosolve. Slightly soluble in benzene and carbon tetrachloride.	4

PREPARATION AND PURIFICATION

1-Methylaminoanthraquinone is prepared by one of several alkylamination reactions. Methylamine is treated with 1-anthraquinonesulfonic acid, 1-chloroanthraquinone, or 1-phenoxyanthraquinone.^{5,6} Yields are high and are about the same for each method. Consequently, the industrial route of synthesis depends on the cost and availability of the starting materials. Impurities may include any of the chemicals used in the preparation procedure as well as dimethylamino derivatives, mercury, copper, boric acid, and possibly anthracene.

Purification can be achieved by recrystallization from benzene or acetic acid³ or carbon tetrachloride.² However, one study shows that melting point and absorption spectra are insensitive to changes in concentrations of impurities below 2%.² Extraction methods have been developed for removal of mercury when it is present in levels below 5 ppm.⁷

PRODUCTION AND USE

In 1973, four small U.S. companies manufactured the dye.⁸ Imports of the dye totaled 54,912 pounds as 1-methylaminoanthraquinone and 6,710 pounds as Solvent Red 111 for that year.⁹ Imports totaled 13,260 pounds as 1-methylaminoanthraquinone and 7,260 pounds as Solvent Red 111 in 1974.¹⁰

1-Methylaminoanthraquinone is used extensively in the textile and paper industry. It is an important intermediate for the synthesis of other dyes. At the present time, it has not been certified for use in cosmetics, foods, or drugs.

CHEMISTRY

1-Methylaminoanthraquinone undergoes the typical reactions of anthraquinones, that is, reduction, sulfonations, nitrations, and halogenations. In addition, the secondary amino group increases the acid solubility and facilitates reactions with weak and strong acids. The compound is sensitive to UV light and may undergo additional changes in the presence of atmospheric oxygen.

ANALYTICAL METHODS

1-Methylaminoanthraquinone and other aminoanthraquinone dyes have been separated by column,^{2,11} paper,¹² and thin-layer chromatography.² Solvent systems used for thin-layer chromatography are benzene saturated with water, water:butanol, water:ethyl acetate, carbon tetrachloride saturated with water, and dioxane:water. The separation of 1-methylaminoanthraquinone on aluminum oxide with hexane:acetone^{2,5} as the developing solvent gave an R_f (x 100) of 86. More recently, high pressure liquid chromatography has

been adapted to the separation, identification, and estimation of 1-methylaminoanthraquinone.¹³ Successful development of this technique promises to provide a rapid method for purification of experimental quantities of 1-methylaminoanthraquinone and other pyrotechnic dyes.

MAMMALIAN TOXICOLOGY

Human Exposures

1-Methylaminoanthraquinone is a skin irritant and sensitizer.¹⁴

Experimental Animals

Griswold et al.¹⁵ reported that 1-methylaminoanthraquinone showed only slight toxicity in female Sprague-Dawley rats that received 10 doses of 500 mg/rat/dose through gastric tube; one dose was given every 3 days for 30 days (total dose 5,000 mg/rat). During a 9-month observation period, there were cystic changes in the kidneys of 14 treated female rats; one rat developed renal hyperplasia. One source reports no skin or eye irritation for rabbits.¹⁶

Biochemistry

No information was retrieved.

Carcinogenicity

Griswold et al.¹⁵ reported that during a 9-month observation period of female Sprague-Dawley rats dosed by gastric tube with a total dose of 5,000 mg of 1-methylaminoanthraquinone per rat, only one rat out of 14 developed tubular adenocarcinoma of the kidney. Because carcinoma of the kidney was observed in the controls as well, the authors concluded that the dye is noncarcinogenic to rats.

Mutagenicity

1-Methylaminoanthraquinone was nonmutagenic when screened for mutagenicity with five Salmonella typhimurium tester strains with and without mammalian microsomal activation.¹⁷

ENVIRONMENTAL CONSIDERATIONS

Behavior in Soil and Water

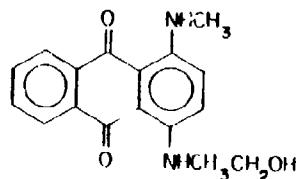
Degradation. No information was retrieved, but it should be noted that anthraquinones are readily reduced to the corresponding diols.

Animals

Mammals. No information was retrieved.

Birds. No information was retrieved.

Fish. No information was retrieved. Concerning related structures, Little et al.¹⁸ have investigated the acute toxicities of a number of anthraquinone dyes to fathead minnows (Pimephales promelas), and the most toxic by far was Disperse Blue 3.



The 96-hour LC₅₀ for Disperse Blue 3 is 1 mg/L at 15°C. Related dyes bearing hydroxyl groups on the aromatic rings are less toxic by several orders of magnitude.¹⁸

Reptiles. No information was retrieved.

Amphibians. No information was retrieved.

Invertebrates. No information was retrieved.

Microorganisms

No information was retrieved.

Plants

No information was retrieved.

Food Chain

No information was retrieved.

EXISTING STANDARDS

No information was retrieved.

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APPENDIX F

VAT YELLOW 4

ALTERNATIVE NAMES

Dibenzo(b,def)chrysene-7,14-dione (C.A. from 1957); dibenzo(a,b)pyrene-7,14-dione (C.A. before 1957); 3,4,8,9-dibenzopyrene-5,10-quinone; 4,5,9,10-dibenzopyrene-3,8-quinone; Indanthrene golden yellow; C.I. Vat Yellow 4; HVT Golden Yellow.

PHYSICAL AND CHEMICAL PROPERTIES

CAS Registry No.: 128-66-5

Colour Index No.: 59100

Toxic Substances List No.: H070300

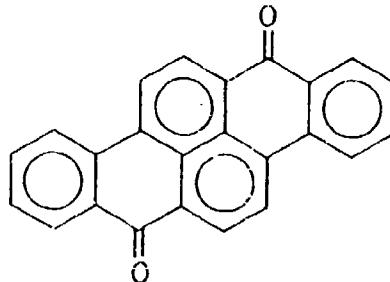
Wiswesser Line Notation: L D6 B66 0666 2AB A JV UV&J

Military Specification: MIL-D-50029C¹

Molecular Weight: 332.3

Empirical Formula: C₂₄H₁₂O₂

Structural Formula:



Physical properties of Vat Yellow 4 are listed in Table F-1.

TABLE F-1. PHYSICAL PROPERTIES OF VAT YELLOW 4

Property	Value	Reference
Melting Point	385°C	2
Boiling Point	-	
Density	-	
Vapor Pressure	-	
Color	Yellow Orange-brown (from nitrobenzene)	2 3
Heat of Sublimation	-	
Solubility in Water	Insoluble	
Solubility in Other Solvents	Soluble in sulfuric acid, tetrahydronaphthalene, xylene, and nitrobenzene; slightly soluble in acetone, benzene, alcohol, chloroform, o-chlorophenol, pyridine, and toluene	4

PREPARATION AND PURIFICATION

Dye Vat Yellow 4 is prepared in 55% or better yield by the following methods: benzoylation of benzanthrone, which is the original synthetic route; ring closure of 1,5-dibenzoylnaphthalene; benzoylation of 1-benzoylnaphthalene followed by ring closure; and direct condensation of naphthalene with benzoylchloride in aluminum chloride at 160°C.^{2,5,6}

Chromatographic purification has been achieved by dissolving Vat Yellow 4 in chlorobenzene and eluting over alumina.⁷ More recent developments have adapted high pressure liquid chromatography techniques to purification and estimation of Vat Yellow 4; when acetonitrile:water (9:10) was used as the developing solvent through a reverse phase column of microporasil C18 at a rate of 1 mL/min and with detector at 280 nm, sensitivity of 200 ng was obtained.⁸ Another method of purification has been to dissolve the dye in concentrated sulfuric acid and to precipitate by pouring the mixture into crushed ice.³ The extremely low solubility of the dye makes it difficult to purify.

PRODUCTION

In 1973, four or more U.S. producers made 98,000 pounds of Vat Yellow 4 dye.⁹ Only 1,162 pounds were imported, all of it as the solubilized dye.¹⁰ In 1974, 1,874 pounds of Vat Yellow 4 as the solubilized dye were produced.¹¹

CHEMISTRY

Vat Yellow 4 is relatively stable, especially to alkaline oxidation media. It is readily halogenated by standard methods. The bromo derivative is prepared by dissolving Vat Yellow 4 in $AlCl_3$ at 160° to 170°C, cooling the mixture, and adding bromine in chlorosulfonic acid. Nitration, hydroxylation, and amination have been done.^{5,12}

ANALYTICAL METHODS

Vat Yellow 4 is determined in batches sold for pyrotechnic mixtures by dissolution in sulfuric acid and measurement of absorbance between 568 and 571 nm. Percent purity is equal to 100 times the ratio of sample absorbance to that of standard, both at 568 and 571 nm.¹³ Vat Yellow 4 is poorly soluble in most solvents and, therefore, the methods for its identification are limited. It has been separated by use of column⁷ and thin-layer chromatography.¹³ The technique of high pressure liquid chromatography has been applied with promising results.⁸

MAMMALIAN TOXICOLOGY

Human Exposures

No information was retrieved regarding human toxicology responses after exposure to Vat Yellow 4. However, Tatyrek¹⁴ indicated that Vat Yellow 4 is toxic and may contain traces of 3,4,8,9-dibenzopyrene (dibenzochrysene), a carcinogen.

Experimental Animals

A single report was retrieved that describes acute oral and dermal toxicity tests using a Vat Yellow 4 paste formulation of unspecified concentration.¹⁵ The acute oral LD₅₀ in male and female rats for this paste exceeded 46 g/kg, whereas the minimum lethal oral dose for male and female rabbits exceeded 11.6 g/kg. Application of the paste to intact and abraded skin of male and female rabbits produced no grossly discernible skin damage, and the acute dermal LD₅₀ for rabbits exceeded 4.6 g/kg. Instillation of the paste into the conjunctival sac of rabbits produced minimal reversible ocular irritation.

Subcutaneous injection or painting of the skin of mice with Vat Yellow 4 over prolonged periods was reported not to result in tumor formation;¹⁶ another paper reports high mortality of mice under these conditions but, again, no tumors.¹⁷

Biochemistry

Tatyrek¹⁴ suggested that it is very unlikely that this Vat Yellow 4 dye can be metabolized by reduction to dibenzopyrene, a carcinogen. There are no published data regarding the metabolic reduction of this dione compound to the carcinogenic dibenzochrysene.

Carcinogenicity

In a study conducted at the Carcinogenesis Studies Branch of the National Cancer Institute (NCI) in 1965, investigators found ca. 0.1% dibenzochrysene, a carcinogen, in a commercial sample of the Vat Yellow 4 dye.¹⁴ There is also a possibility that dibenzopyrene (dibenzochrysene) is formed during the burning of the dye (during smoke formation); the presence of the latter compound in amounts as little as 0.01% would impart carcinogenicity.¹⁴

A commercially formulated material attested by the manufacturer to contain 18.2% of the color-imparting component, 30.8% sorbitol, 5.5% dispersant (Lomar TWC), 2.7% glycerine, and 42.8% water was tested in the NCI Bioassay Program. A summary of their results follows:¹⁸

"A bioassay of C.I. Vat Yellow 4, a commercial formulation containing dibenzo(b,def)chrysene-7,14-dione, for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and B6C3F1 mice.

"Groups of 50 rats of each sex and 50 mice of each sex were administered C.I. Vat Yellow 4 in the diet at one of two doses: either 3,500 or 7,000 ppm for the rats, either 25,000 or 50,000 ppm for the male mice, and either 12,500 or 25,000 ppm for the female mice. The rats were administered the test chemical for 104 weeks; the mice, for 106 weeks. Matched controls consisted of 20 untreated rats and 20 untreated mice of each sex. All surviving animals were killed at the end of the period of administration of the test chemical.

"Mean body weights of the dosed rats were lower than those of corresponding controls throughout the bioassay, but the differences in weights were slight for the males. Mean body weights of the dosed mice were not affected by the test chemical. Survival of the rats and mice was not affected adversely by the chemical, and sufficient numbers of dosed and control rats and mice of each sex were at risk for the development of late-appearing tumors.

"In the male and female rats and the female mice, no tumors occurred at incidences that were significantly higher in dosed groups than in control groups.

"In the male mice, lymphomas occurred at incidences that were dose related ($P = 0.002$), and, in a direct comparison, the incidence of the tumor in the high-dose group was significantly higher ($P = 0.019$) than that in the control group (controls 3/20, or 15%; low-dose 7/47, or 15%; high-dose 22/50, or 44%). The incidence of lymphomas and leukemias in historical-control male B6C3F1 mice at this laboratory was 38/323 (12%).

"It is concluded that under the conditions of this bioassay, the formulated product containing C.I. Vat Yellow 4 was not carcinogenic for male or female Fischer 344 rats or for female B6C3F1 mice, but was carcinogenic for male B6C3F1 mice, causing an increased incidence of lymphomas."

However, because chemical analysis of the test material was not performed, the observed increased incidence of lymphomas in male mice dosed with the formulated product cannot be attributed with complete confidence to Vat Yellow 4.

ENVIRONMENTAL CONSIDERATIONS

Behavior in Soil and Water

No information was retrieved.

Animals

Mammals. No information was retrieved.

Birds. No information was retrieved.

Fish. No information was retrieved. However, Little et al. report that for the fathead minnow the 96-hour LC₅₀ for the dibromo derivative, Vat Orange 1, exceeds 180 mg/L at 15°C.¹⁹

Reptiles. No information was retrieved.

Amphibians. No information was retrieved.

Invertebrates. No information was retrieved.

Microorganisms

No information was retrieved.

Plants

No information was retrieved.

Food Chain

No information was retrieved.

EXISTING STANDARDS

No information was retrieved.

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APPENDIX G

HEXACHLOROETHANE

ALTERNATIVE NAMES

Hexachloroethane; 1,1,1,2,2,2-hexachloroethane; Avlothane; Ethanehexachloride; Fasciolin(e); Hexoram; Perchloroethane; Phenohep.

PHYSICAL AND CHEMICAL PROPERTIES

CAS Registry No.: 67-72-1¹

Colour Index No.: None

Toxic Substances List No.: KI40250¹

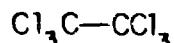
Wiswesser Line Notation: GXCGXGGG¹

Military Specification: MIL-H-235B²

Molecular Weight: 236.70

Empirical Formula: C₂C₁₆

Structural Formula:



Physical properties of hexachloroethane are summarized in Table G-1.

PREPARATION

Small quantities of highly pure hexachloroethane may be prepared by the action of chlorine on barium carbide. Jondorf and co-workers⁶ used this procedure to make ¹⁴C-labeled hexachloroethane. Hexachloroethane is formed when lower normal hydrocarbons or chlorinated hydrocarbons are allowed to react with excess chlorine in the presence of ultraviolet light⁷ or in the presence of chlorination catalysts at temperatures above 200°C.⁸ In the usual industrial process, tetrachloroethylene is chlorinated in the presence of ferric chloride, at 100° to 140°C, in a lead-lined vessel.⁹ Hexachloroethane is also obtained as a coproduct in the production of tetrachloroethylene by pyrolysis of carbon tetrachloride at 800° to 900°C.⁹ A process in which a mixture of ethylene and chlorine is passed over charcoal at 300° to 350°C is reported to give hexachloroethane yields of 80 to 90%.¹⁰

TABLE G-1. PHYSICAL PROPERTIES OF HEXACHLOROETHANE

Property	Value	Reference
Melting Point	186.9°-187.4°C (sealed tube); Sublimes without melting	3 3,4
Boiling Point	186.8°C 184.4°C	4 5
Density	2.091 g/ml	3,4
Vapor Pressure	1 mm Hg (20.0°C) 3 mm Hg (32.7°C)	3 4
Color	White	4
Odor	Camphoraceous	4
Crystalline Form	Rhombic	3
Heat of Sublimation	12.2 kcal/mole	4
Solubility in Water	50 mg/kg (22°C)	5
Solubility in Other Solvents	Soluble in alcohol, benzene, chloroform, ether, and oils	4

PRODUCTION AND USE

In addition to its military use for generating screening smokes,¹¹ hexachloroethane has found some application in veterinary medicine for treatment of intestinal worms. Most material in current use appears to have been imported. Parker et al. reported that 760,000 kg were imported in 1976.¹²

CHEMISTRY

Hexachloroethane, unlike most of the chloroethanes, is stable in alkaline media.³ Hexachloroethane presents a slight explosion hazard through spontaneous chemical reaction. Dehalogenation by alkalis, metals, etc., will produce spontaneously explosive chloroacetylenes.¹³ At high temperatures, especially in the presence of moisture, hexachloroethane decomposes with a corrosive attack on metals.³

Sjoberg¹⁴ has studied the decomposition of hexachloroethane vapor at 300° to 500°C in the presence of metal filings to produce phosgene and hydrogen chloride. The maximum yield of 6 mg of phosgene and 49 mg of hydrogen chloride per gram of hexachloroethane was observed at 450°C in the presence of iron. (Carbon tetrachloride, by comparison, gives 80 mg of phosgene per gram under the same conditions.) Over glowing charcoal, hexachloroethane produced 2 to 10 mg of phosgene per gram and as much as 474 mg of hydrochloric acid.¹⁴ In the gas phase, oxidation of hexachloroethane proceeds via thermal dissociation with subsequent phosgene formation.¹⁵

Mixtures for production of pyrotechnic white smoke in British smoke generators contain 46% hexachloroethane, 29 to 47% zinc oxide, and 7 to 25% calcium silicide, the fuel.¹⁶ Burning temperatures range from 700° to 1,100°C. Jarvis¹⁶ has found that hexachloroethane is pyrolyzed to carbon and chlorine via intermediate formation of carbon tetrachloride, tetrachloroethylene, and hexachlorobenzene. Calcium silicide reacts with hexachloroethane in stages to form calcium chloride, carbon, and silicon. Zinc oxide also reacts stepwise to produce zinc chloride (the smoke), carbon monoxide, and carbon dioxide; intermediates include phosgene and zinc oxychloride in addition to chlorocarbons.

ANALYTICAL METHODS

Current methods for detection and quantitation of chlorocarbons in public water supplies variously employ sorption on activated carbon¹⁷⁻²⁰ or macroreticular resin^{17,19-21} and desorption, or continuous liquid-liquid extraction,²² combined with gas chromatography using flame ionization,^{19,20} electron capture,¹⁸ or mass spectrometric^{17,18,21-25} detection for identification of hexachloroethane. Keith et al.¹⁷ have used computerized gas chromatography/mass spectrometry for detection of hexachloroethane after concentration on XAD-2 resin or carbon column extraction. A detection limit of 0.03 µg/L is

reported. Others have noted that carefully prewashed carbon gives better recovery of hexachloroethane than XAD resins.^{19,20} In either case, the chlorocarbon typically is desorbed using acetone and chloroform and concentrated by means of a Kuderna-Danish evaporator. Although detection limits as low as 0.01 $\mu\text{g/L}$ have been reported,²⁰ quantitation of trace levels of hexachloroethane is complicated by low recoveries from water at these levels. Hexachloroethane has also been detected in wastewater by extraction with pentane followed by direct injection of the extract onto a tritolylphosphate-coated gas chromatography column with a flame ionization detector.²⁴ A more cumbersome method, but one that avoids the requirement for gas chromatography equipment, involves sorption on activated carbon and elution with a nonhalogenated solvent followed by conversion of the chlorocarbon to inorganic chloride, which is titrated with silver nitrate solution.²⁵

Hexachloroethane has been separated from biological specimens by steam distillation and identified by microcrystallography,²⁶ with as little as 20 mg hexachloroethane per 100 g of the biological specimen being assayed. It has also been detected in biological tissues at levels below 1 ppm by gas chromatographic methods.²⁷

Hexachloroethane, present in relatively high concentrations, has been separated by distillation from mixtures of chloroethanes and estimated refractometrically²⁸ with 0.5 to 2.0% accuracies. Both infrared and mass spectroscopic techniques can be used to detect hexachloroethane and other chlorohydrocarbons in chloroform.²⁹ By using infrared spectroscopic analysis, Fisher et al.³⁰ were able to determine the presence of hexachloroethane in a two component mixture of chlorinated hydrocarbons with a relative precision of $\pm 25\%$. The band at 14.65μ was used to distinguish hexachloroethane.

Small quantities of hexachloroethane can be estimated by passing air over vapors from a hot aqueous solution of chlorine-containing material through quartz tubes at 900°C to give carbon dioxide and hydrogen chloride. The hydrogen chloride is estimated volumetrically by titration with mercuric nitrate using diphenylcarbazide/bromphenol blue as the indicator. The average error of analysis is $<2\%.$ ³¹

A sampling and analysis method for hexachloroethane in air (S101) is described in the NIOSH Manual of Analytical Methods.³²

MAMMALIAN TOXICOLOGY

Human Exposures

Parker et al.¹² have estimated (1979) that 1,500 workers are exposed to hexachloroethane; these include cleaners and charwomen, millwrights, machine operatives, plumbers, pipefitters, and electricians. Von Oettingen reported in 1955 that little was known regarding the toxicity of hexachloroethane to man.⁵ The inhalation hazard may be low because of the high boiling point of hexachloroethane, but cutaneous exposure should be viewed as a potential hazard.

Hexachloroethane is moderately irritating to the skin, mucous membranes, and liver. In high concentrations, it is narcotic.¹ It may be absorbed through the gastrointestinal tract, the lungs, and the skin.³³ In 1947, Plotnikov ingested 30 g hexachloroethane in 3 days and Sokolov 48 g in 4 days without any harm; only skin sensitivity was somewhat diminished.³⁴ Saric and Knezevic reported in 1957 that irritation was the most frequent complaint of workers exposed to hexachloroethane in dry cleaning and drug factories, but serious symptoms were scarce.³⁵ The serum albumin:globulin ratio changed, but no other sign of liver injury was apparent.

Fischer reported that 70 persons were injured and 10 persons died in Malta in 1943 following exposure to smoke grenade fumes in a tunnel.³⁶ He mentioned that serious accidents occasionally occurred among workers who handled smoke chemicals containing hexachloroethane. The profound pathological changes in the lungs, livers, and kidneys of the victims were attributed, however, to the toxicity of zinc chloride, the major ingredient of the smoke mixture.

Hexachloroethane lone is not considered a significant industrial hazard if handled with reasonable care.³⁷ Hexachloroethane when inhaled, ingested, or absorbed by the skin can cause acute local changes. Inhalation may also cause acute changes that affect general bodily functions. These changes may be "irreversible (or) reversible but are not severe enough to cause death or permanent injury."¹⁰ A chronic local condition producing these same changes can develop when hexachloroethane is absorbed by the skin. Eye irritation, tearing of eyes, inflammation of eye membrane, photophobia, and inability to close eyelids have been noted as consequences of hexachloroethane exposure.¹²

Experimental Animals

Parker et al.¹² have summarized toxic effects of hexachloroethane on experimental animals. Acute toxicity studies indicate a range of lethal doses that depend on the route of administration and the test species employed. The lowest lethal dose of hexachloroethane administered subcutaneously to rabbits has been 4 g/kg.³⁸ Plotnikov and Sokolov reported in 1947³⁴ that the lethal dose of hexachloroethane administered orally in capsules or subcutaneously in oil to cats was 6 g/kg. Cats and dogs were given total doses up to 18 g/kg in two or four courses of 2 days each; only one of the six cats died and all six dogs survived. The chief symptoms were central nervous system effects, narcosis, and fatty liver degeneration.³⁴

Barsoum and Saad³⁹ found that 0.325 g/kg administered intravenously killed dogs in 30 minutes. Death was due to respiratory failure. Tracheotomy and artificial respiration resulted in extended survival times. The post-mortem examination indicated fatty degeneration of the liver. The dose needed to cause death in dogs within 30 minutes was 0.10 g/kg for pentachloroethane and 0.09 g/kg for chloroform. Oral doses of hexachloroethane as high as

6 g/kg produced no mortalities. Maloff found that the fat content of the liver of an 11.6-kg dog was essentially unchanged by administration of 82 g of hexachloroethane in 1- to 2-g daily oral (encapsuled) doses and 10 g in 1-g daily subcutaneous doses.³⁹

Weeks et al.⁴⁰ studied the acute toxicity of hexachloroethane using a variety of test animal species and routes of administration. In acute oral toxicity studies, the test compound was dissolved in corn oil (50% weight/volume) or methylcellulose (5% weight/volume). Solutions were administered by stomach tube (0.1 mL/10 g body weight) to male and female rats, male guinea pigs, and male rabbits. The acute oral and dermal approximate lethal dosage (ALD) and dermal LD₅₀ for rabbits and the oral LD₅₀ for guinea pigs and rats were calculated on the basis of survival for a 14-day observation period. These values are shown in Table G-2.

Schwander⁴¹ and Burgi⁴² studied diffusion of hexachloroethane through the abdominal skin of rabbits. Hexachloroethane, dissolved in carbon tetrachloride, appeared in the exhaled air 40 minutes following application to the skin. At 6 hours after application, the corneal reflex was intact, but the animals were apathetic and did not recover from hard breathing after 5 days. In the absence of a solvent, hexachloroethane appears to be relatively nontoxic when applied to skin.

Chronic toxicity studies were performed with rabbits.⁴⁰ Daily oral doses of hexachloroethane suspended in 5% aqueous methylcellulose (1,000, 320, or 100 mg/kg) were given for 12 days to groups of five male rabbits to monitor the development of toxic signs and changes in blood chemistry values. Daily oral dosages of 1,000 mg/kg caused a significant ($P < 0.05$) reduction in body weight beginning at day 7 of dosing. Increases in liver and kidney organ-to-body weight ratios were noted at necropsy ($P < 0.05$); no other changes were found at necropsy. The 320-mg/kg dosages caused a significant ($P < 0.05$) reduction in body weight gain beginning at day 10, but there were no toxic signs and no changes in organ-to-body weight ratios at necropsy. A dosage of 100 mg/kg/day produced no changes in the criteria studied. The only blood chemical parameters affected were the potassium and glucose values, which decreased significantly at the 1,000- and 320-mg/kg dosage levels.

Reaves and Carlon⁴³ indicated in 1961 that the toxic effects of smoke grenades (containing hexachloroethane and zinc oxide) to dogs, rabbits, and rats were due to zinc chloride. The damage was localized in the respiratory system and was manifested by chronic degeneration of the tissues.

Weeks et al.⁴⁰ report data from an extensive series of vapor inhalation studies in male and female rats with separate tests for acute toxicity, subchronic toxicity, behavioral responses, pulmonary function, and oxygen consumption. Exposure to a nominal concentration of 2.5 mg/L (260 ppm) for 8 hours showed no toxic signs during exposure or for 14 days thereafter. Exposure to a nominal concentration of 57 mg/L (5,900 ppm) for 8 hours showed severe toxic signs including death. At 8 hours, two of six rats were dead. Surviving rats showed reduced body weight gain over the 14-day observation period compared with controls.⁴⁰

TABLE G-2. ACUTE TOXICITY OF HEXACHLOROETHANE⁴⁰

Animal	Treatment	Viluent	Value (mg/kg)
Rabbit, male	Oral ALD	Methylcellulose	>1,000
Rat, male	Intraperitoneal ALD	Corn oil	2,900
Rat, female	Oral LD ₅₀	Corn oil Methylcellulose	4,460 7,080
Rat, male	Oral LD ₅₀	Corn oil Methylcellulose	5,160 7,690
Guinea pig, male	Oral LD ₅₀	Corn oil	4,970
Rabbit, male	Dermal LD ₅₀	Water paste	>32,000

The subchronic inhalation toxicity of hexachloroethane vapor was studied in male and female rats, male dogs, male guinea pigs, male quail, and pregnant rats. The groups and numbers of animals exposed and the analytical air concentrations of hexachloroethane are shown in Table G-3.

The subchronic inhalation hazard for hexachloroethane vapor was tested by exposing animals to control air and three concentrations of hexachloroethane for 6 hours a day, 5 days a week for 6 weeks.⁴⁰ Chamber air samples for all concentrations were analyzed by gas chromatography. All animals used in these experiments were observed during a preliminary period, and control groups exposed to chamber air were matched with each treatment group in respect to number, age, sex, and body weight.

Dogs exposed to 250 ppm hexachloroethane developed tremors, were ataxic, hypersalivated, showed severe head bobbing and facial muscular fasciculations, and held their eyes closed during the exposure. One dog convulsed during the first exposure and died after 5 hours of exposure.⁴⁰

Guinea pigs exposed to 260 ppm hexachloroethane showed a reduction in body weight gain beginning the second week of exposure. Two guinea pigs died during each of the fourth and fifth weeks. At necropsy, the liver-to-body weight ratio was significantly higher than the ratios in controls.⁴⁰

Body weight gain of the male exposed to 260 ppm but not the nonpregnant female rats was reduced starting with the third week of exposure.⁴⁰ All rats showed tremors, ruffled pelt, and red exudate around the eyes following exposure during the fourth week, and one male and one female rat were found dead. At the end of the exposure period, all signs disappeared and the body weight gain reflected that of the controls. The kidney-, spleen-, and testes-to-body weight ratios in the male rat and the liver ratios in the female rats were significantly larger than controls. The older rats (12 to 14 weeks, 300 to 350 g) in the behavior group showed no toxic signs during exposure. The body weight gain was less than controls, and at necropsy the lung-, liver-, kidney-, and testes-to-body weight ratios were increased over controls. Fatty infiltration of the kidney was seen at necropsy.

Quail exposed to 260 ppm hexachloroethane showed no adverse signs, no effects on body weight, and no gross organ or tissue changes at necropsy.⁴⁰ All animals sacrificed 12 weeks after termination of exposure showed no gross changes in tissues and organs. Body weight changes of all animals were comparable to the controls.

In behavior tests, hexachloroethane produced no measurable effects on either avoidance performance or spontaneous motor activity in rats.⁴⁰ Hexachloroethane is moderately narcotic.⁴⁴ Steindorff⁴⁵ observed that hexachloroethane has paralytic effects on the nervous system of the dog, and Binz⁴⁴ found that oral doses of 1 to 1.1 g/kg in dogs caused depression of the central nervous system characterized by weakness, staggering gait, and twitching of the muscles.

TABLE G-3. EXPOSURE OF VARIOUS ANIMALS TO HEXACHLOROETHANE (HCE)⁴⁰

Treatment	Pats		Behavior Rats	Pregnant Rats	Dogs	Guinea Pigs		Quail
	Male	Female						
Control (air only)	25	25	15	22	4	10	20	
15 ppm HCE	25	25	15	21	4	10	20	
48 ppm HCE	25	25	15	22	4	10	20	
260 ppm HCE	25	25	15	22	4	10	20	

Although dogs exposed to 260 ppm hexachloroethane showed severe signs of irritation, no changes in pulmonary function could be detected.⁴⁴ Six-week exposure to 260 ppm hexachloroethane produced a significant decrease in oxygen consumption.⁴⁰ The test was nonspecific, but indicated an alteration in general metabolism due to hexachloroethane exposure. This change in the absence of other supportive pathology does not indicate a serious health hazard, but may be a normal response to the inhalation of an upper respiratory irritant.

Irritation responses to hexachloroethane were studied by eye and skin application tests.⁴⁶ Crystalline hexachloroethane (0.1 g) applied to the corneas of rabbits and allowed to remain overnight caused moderate corneal opacity, iritis, and severe swelling and discharge in 5 of 6 rabbits. No signs were seen 72 hours after administration. Hexachloroethane had no toxic effect on the eyes of dogs.⁴⁵

The potential for primary skin irritation was tested by 24-hour application of 0.5 g of either the dry crystalline material or of a water paste to the intact and abraded skin of six rabbits.⁴⁰ The dry material caused no skin irritation, and the paste caused only slight redness that disappeared after 72 hours.

A 3-week inhalation exposure (260 ppm) period with a 2-week rest and then challenge with one dose of sensitizing solution (0.1 mL of 0.1% hexachloroethane in propylene glycol and saline) showed that hexachloroethane produced no recognizable sensitization reactions in a group of 10 male guinea pigs. However, dinitrochlorobenzene (a known sensitizer) produced definite sensitization reactions in 10 out of 10 animals.⁴⁶

Teratogenicity studies in rats indicate that hexachloroethane at dosages toxic to dams (500 mg/kg oral or 260 ppm by inhalation) did not produce a teratogenic effect.⁴⁰ High dosages of hexachloroethane that were toxic to the dams resulted in a slight slowing of fetal development.

Studies to determine the potential of hexachloroethane for inducing liver enzyme formation were conducted using three groups of 10 male rats.⁴⁰ At an intraperitoneal dose of 500 mg/kg dissolved in corn oil per day for 4 consecutive days, hexachloroethane did not induce hepatic microsomal enzymes. Data reporting organ-to-body weight changes in subchronic studies are not consistent with the results of this experiment.

Biochemistry

Jondorf et al.⁶ reported in 1957 that rabbits slowly metabolize hexachloroethane. In in vivo studies indicate most metabolites are excreted in the feces and in exhaled air (about 14 to 24%), with only small amounts excreted in the urine (about 5%).⁶ Metabolic studies in various animals confirm the formation of 12 metabolites: three possible metabolites (trichloroethylene, monochloroethanol, and acetate) have not been detected (Table G-4).

TABLE G-4. LIST OF METABOLITES OF HEXACHLOROETHANE

Metabolite	Organism or Test System	Reference	Comments
Substances Detected			
hexachloroethane	Rabbit	6,7,14,42	0.5% in exhaled air appeared 4.0 minutes after application of hexachloroethane (dissolved in carbon tetrachloride) to the skin
Pentachloroethane	Heptatic microsomal enzymes of sheep and rats	46,47	Main metabolite, produced by dechlorination of hexachloroethane
Pentachloroethane	Pow. duck, dog, cockerel	48,49	Main metabolite
1,1,1-trichloroethane	Rabbit	6	0.6% in exhaled air
1,1,2,2-tetrachloroethane	Rabbit	6	Evidence for this metabolite suggested by <u>in vivo</u> metabolism studies
Dechlorinated hexachloroethane	Heptatic enzyme system of rabbit, rat, and mouse	47,50	<u>In vitro</u> determination requiring optimum pH of 8.2 and NADPH and oxygen
Pentachloroethylene	Rabbit	6	12.0% in exhaled air
Pentachloroethylene	Heptatic microsomal enzymes of sheep and rats	46,47	Main metabolite produced by dechlorination of hexachloroethane

TABLE 3-4 (Cont.)

Metabolite	Organism or Test System	Reference	Comments
Tetrachloroethylene	Fowl, duck	48,49	Main metabolite
Trichloroethanol	Rabbit	5	1.3% in urine
Dichloroethanol	Rabbit	6	6.4% in urine
Trichloroacetic Acid	Rabbit	6	1.3% in urine
Dichloroacetic Acid	Rabbit	6	0.8% in urine
Monochloroacetic Acid	Rabbit	6	0.7% in urine
Oxalic Acid	Rabbit	6	0.1% in urine
Carbon Dioxide	Rabbit	6	0.3% in exhaled air
<u>Substances Not Detected</u>			
Trichloroethylene	Rabbit	6	None detected in exhaled air
Monochloroethanol	Rabbit	6	None detected in urine
Acetate	Rabbit	6	None detected in urine

Reynolds and Yee⁵¹ found that hexachloroethane did not cause early centrilobular suppression of glucose-6-phosphatase activity in the liver of rats, in contrast to carbon tetrachloride. There was only slight depression of enzyme activity, indicating transient slight liver damage. Intragastrically administered hexachloroethane (2,440 mg/kg) highly inhibited rat liver microsomal hydroxylation reactions after 24 hours, but had no effect on epoxide hydratase activity.⁵² Fed intragastrically to rats at 6,150 mg/kg, hexachloroethane was reported to be without effect after 2 hours on hepatic microsomal functional properties, including protein content, oxidative demethylase, glucose-6-phosphatase, NADPH-NT reductase, ¹⁴C-glycine incorporation, cell sap RNA, and lipid conjugated diene content.⁵³

In sheep, the plasma level of sorbitol dehydrogenase, glutamate dehydrogenase, and ornithine carbamoyl transferase increased, indicating cell membrane permeability changes.⁴⁶ The bromosulfophthalein dye clearance was retarded, indicating liver damage due to hexachloroethane toxicity.⁴⁶ Fowler⁴⁸ found a decrease in the bromosulfophthalein dye clearance in ducks and cockerels fed hexachloroethane through stomach tubes, as well as an increase in the level of aspartate aminotransferase.

Carcinogenicity

Hexachloroethane was tested in the NCI Bioassay Program under C04604. A summary of the results follows.⁵⁴

"A bioassay for possible carcinogenicity of technical-grade hexachloroethane was conducted using Osborne-Mendel rats and B6C3F1 mice. Hexachloroethane in corn oil was administered by gavage, at either of two dosages, to groups of 50 male and 50 female animals of each species. The chemical was administered 5 days a week, cyclically for 44 of 78 weeks in rats and continuously for 78 weeks in mice, followed by an observation period of 33 or 34 weeks for rats and 12 or 13 weeks for mice. The high and low time-weighted average dosages of hexachloroethane were, respectively, 423 and 212 mg/kg/day for male and female rats and 1179 and 590 mg/kg/day for male and female mice. For each species, 20 animals of each sex were placed on test as vehicle controls. These animals were gavaged with pure corn oil at the same rate as the high dose group of the same sex. Twenty animals of each sex were placed on test as untreated controls for each species. These animals were not intubated.

"A statistically significant association between increased dosage and accelerated mortality was observed in male and female rats but not in mice of either sex.

"Toxic tubular nephropathy was observed in all groups of treated animals.

"Statistical evaluation of the incidences of hepatocellular carcinomas revealed a significant positive association between hexachloroethane

administration and tumor incidence in both male and female mice. No statistical significance was attributed to the incidence of any neoplasia in rats of either sex.

"No evidence was provided for the carcinogenicity of the compound in Osborne-Mendel rats. It is concluded that under the conditions of this bioassay, hexachloroethane was carcinogenic in B6C3F1 mice, inducing hepatocellular carcinomas in both sexes."

Mutagenicity

Mutagenicity screening tests (Saccharomyces cerevisiae and Salmonella typhimurium) were performed with hexachloroethane over a series of concentrations such that there was either quantitative or qualitative evidence of some physiological effect at the high dose level.⁴⁰ The low dose was, in all cases, below a concentration that demonstrated any toxic effect. Dimethyl sulfoxide was used to prepare 2.5% stock solutions of this compound. The dose range for the evaluation of this compound was from 0.1 to 500 g per plate. Positive and solvent controls using both active mutagens and those that require metabolic activation were run with each assay. The results of the tests conducted in the absence or the presence of the rat liver activation were all negative.

ENVIRONMENTAL CONSIDERATIONS

Behavior in Soil and Water

Degradation. No information is available concerning the persistence of hexachloroethane in soil or water. The vapor pressure³⁹ (1 mm at 20°C) allows one to conclude that hexachloroethane spilled on soil would gradually sublime. The water solubility⁴¹ (50 mg/kg) indicates that leaching by precipitation, surface waters, and groundwater could occur.

Animals

Mammals. Parasivuk⁵⁵ observed that 0.1 g/kg administered orally to horses did not cause noticeable toxic effects and that 0.04 to 1 g/kg stimulated red and white blood cell formation. On the other hand, 6 g/kg caused depression, loss of appetite, and gasping for air. With doses of 7 to 10 g/kg, these effects were exaggerated, and 10 g/kg caused irreversible immo-

In 1955, bywater⁵⁶ mentioned that some toxic effects were observed during the antihelminthic treatment of cattle by hexachloroethane, and death occurred in about 1.2% of the treated animals. Ehrlich and Winterhalter⁵⁷ reported that hexachloroethane produced chronic liver lesions and acute diffuse nephrosis and digestive disorders in cattle. However, Tapernoux⁵⁸ reported in 1930 that 10-g doses of hexachloroethane were well tolerated by the animals; Olsen^{59,60} found in 1944 that cattle tolerated 100 g of

hexachloroethane well, whereas twice this dose caused no ill effect if the cattle were in average condition. De Meek and Baudet⁶¹ found that hexachloroethane is less toxic to cattle than carbon tetrachloride.

Southeast⁶² reported in 1951 that 13.5 g given orally to Merino sheep induced toxic effects. The symptoms included inability to rise, central nervous system depression; staggering walks; fine tremors in the muscles of the lips, face, neck, and forelegs; weak but slightly accelerated pulse; and shallow but otherwise normal breathing. Endrejat⁶³ described the toxic effect of hexachloroethane on sheep following parasite treatment. Kone⁶⁴ found that young sheep exhibited significant decrease in glucose tolerance due to hexachloroethane treatment.

Birds. No information was retrieved other than the studies of Weeks et al.⁶⁵ with quail and of Fowler⁶⁶ with domestic birds.

Fish. No information was retrieved.

Reptiles. No information was retrieved.

Amphibians. No information was retrieved.

Invertebrates. Hexachloroethane vapors are insecticidal. A 2:1 mixture of hexachloroethane and talc spread on water (dosage unknown) reportedly destroyed mosquito larvae (Culex, Anopheles, Aedes, and Stegomyia) without killing other aquatic organisms.^{65,66} Hexachloroethane is described as only moderately toxic to the ova, larvae, and pupae of Musca domestica and Musca vicinia.⁶⁷ Hexachloroethane-carbon tetrachloride mixtures are toxic to Tribolium castaneum (common flour beetle);⁶⁸ and control of the corn earworm, Heliothis obsoleta, by fumigation with hexachloroethane has been reported.⁶⁹ An aqueous solution containing 5 to 10 mg/L of hexachloroethane reportedly caused a multiple discharge nerve axon response when applied to the isolated chela of crayfish (Cambarus virilis).⁷⁰ The same response is elicited by DDT at much lower concentrations.

Several reports describe the use of hexachloroethane for treatment of cattle,^{59,60} sheep,^{60,71} goats,⁷² and white-tailed deer⁷³ infested by parasitic worms, in particular the liver fluke, Fasciola hepatica,⁷⁴ and the stomach worm, Haemonchus contortus.⁶² In vitro studies by Mackie and Parneil⁷⁵ indicate that 45-minute exposure to 1,000 mg/L of hexachloroethane is lethal to F. hepatica, whereas 1,200 mg/L killed 90% of free-living stages of sclerostomes. Bartlett, on the other hand, reported that hexachloroethane has little effect on mature F. hepatica flukes in vitro, but that pentachloroethane, a metabolite in sheep, is twice as potent as carbon tetrachloride as a spasmogen for F. hepatica.⁷¹ F. hepatica recovered from rats and rabbits treated with hexachloroethane showed that spermatogenesis was significantly disrupted.⁷⁶ Hexachloroethane at a concentration of 10 to 100 mg/L was found to be lethal to the protoscoleces of Echinococcus multilocularis incubated in vitro for 2 to 10 days.⁷⁷ At oral doses of 2,600 and 3,500 mg/kg,

hexachloroethane was reported to kill 50 and 100%, respectively, of subcutaneously implanted adult Fasciola in rats.⁷⁸ Hexachloroethane is ineffective against the small flukeworms, Dicrocoeliose ovine,⁷⁹ and some other parasitic worms, Oesophagostomum columbianum and Trichostrongylus spp.⁸⁰ Tsaturyan⁸⁰ reported that hexachloroethane is used successfully in the treatment of louse infection.

Microorganisms

An agar slant culture of Vibrio Metschnikoff (V. metchnikovii?) was reported destroyed in 24 hours by an aqueous solution containing 5.3 mg/l hexachloroethane.⁸¹

Plants

No information was retrieved.

Food Chain

No information was retrieved.

EXISTING Standards

The threshold limit value (for skin only) as given by the American Conference of Governmental Industrial Hygienists is 1 ppm (approximately 9.1 mg/m³). Because of the paucity of reports of human experience, it is not known whether this threshold limit value is sufficiently low to prevent toxic injury in all cases.⁸²

The Environmental Protection Agency has proposed a limit of 47 µg/day* in hexachloroethane, calculated to keep the lifetime cancer risk below 10⁻⁵ for a 70-kg human.⁸³

In proposed limits for water aquatic life, the Environmental Protection Agency has proposed a 24-hour average hexachloroethane concentration of 62 µg/L not to exceed 14 µg/L at any time; for saltwater aquatic life, the 24-hour average hexachloroethane concentration of 16 µg/L, not to exceed 16 µg/L at any time.⁸⁴

*The cancer risk is determined by a linearized one-hit slope. $\delta_H = 9.01 \times 10^{-6}$ day⁻¹ dm⁻³, based on animal carcinogenicities.

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